TITLE: UNIVERSAL PRIMERS FOR WILDLIFE IDENTIFICATION

FIELD OF THE INVENTION

The invention relates to the identification of novel universal primers that can amplify the fragment of cytochrome b gene of any animal species in polymerase chain reaction (PCR) and reveal the identity of the biological material of any unknown animal origin at species and sub-species sources. The invention also provides a method for the identification of fragments on mitochondrial cytochrome b gene in biological material of unknown origin.

BACKGROUND & PRIOR ART REFERENCES

A large number of studies in evolutionary biology utilize phylogenetic information obtained from mitochondrial cytochrome b gene. It has been identified a potent molecule to distinguish the phylogenetic depth of different lineages to family, genus and species in molecular taxonomy[1-66]. A vast database of the sequences of cytochrome b gene of different animal species has accumulated in public databases such as GenBank, NCBI (http://www.ncbi.nlm.nih.gov) etc. We have utilized this capacity of cytochrome b gene in establishing the identity of the origin of animal parts and product to its family, genus and species sources. The technique developed is based on a pair of universal primer that can amplify a small fragment of cytochrome b gene from a vast range of animal species.

Establishing identity of confiscated animal parts and products is a great challenge to law enforcement agencies because none of the methods available till date is too efficient to reveal the identity of animal remains beyond a reasonable doubt. Morphological markers, described for certain species allow the identification of complete specimen of animals[67.] However, a complete specimen is confiscated very rarely by the investigation agencies; therefore, these marker are not practical in wildlife forensics. The biochemical traits such as the bile characteristics[68] blood heam analysis[69,70] etc. have also been employed in wildlife forensic for identification of individual species. The difficulty of these markers are that these markers are limited in number and are rarely found in their natural forms in which these were originally described as the characteristic of a particular species.

The molecular approaches such as micro-satellite based identification[71], Restriction fragment length polymorphism analysis of mitochondrial   genes or   PCR based species specific STS markers require the prior information of the species to   establish the

identity[72,73]. These methods also need a significant amount of DNA material to be analysed. We may not have the prior information about the species origin of confiscated animal parts and product in forensics, therefore, these methods are not really useful and practical in wildlife identification. The technique invented by us is universal, therefore does not require any background information to establish the identity of any unknown confiscated remains at family, genus and species sources. Being a PCR based procedure it can be applied with trace amount of any biological material. Because the amplicon length is small (472 bp); therefore, it can work perfectly with the mutilated remains, which are commonly seized by the crime investigation agencies. It does not require the large amount of genetic material i.e. DNA to be analyzed to establish the identity, hence, can detect a minute amount of adulteration in food products. The procedure described is simple and very fast. Due to the said advantages, the procedure invented by us is most suited for forensic wildlife identification.

OBJECTS OF THE INVENTION

The main object of the invention is to identify a fragment on mitochondrial cytochrome b gene capable of significantly discriminating among various evolutionary lineages of different animal species.

Another object is to identify a fragment on mitochondrial cytochrome b gene which is flanked by the highly conserved sequences at a vast range of animal species.

Yet another object is to detect a fragment on mitochondrial cytochrome b gene which is polymorphic inter-specifically, but monomorphic at intra species sources.

Still another object is to develop the universal primers to amplify the fragment on mitochondrial cytochrome b gene using polymerase chain reaction.

Another object is to develop a PCR protocol that works universally with DNA template of any unknown origin (i.e. all the animal species).

Yet another object is to provide a universal method for identification of species of analyzed material (i.e. the DNA isolated from confiscated animal remain of unknown origin) using the public databases such as GenBank, NCBI etc.

Still another object is to provide a universal method of animal identification to establish the crime with the criminal beyond a reasonably doubt.

Another object is provide a universal method to establish the identity of biological materials such as skin, horns etc confiscated from animal poachers, if it is that of an endangered species.

Yet another object is to provide a universal method for establishment of the identity of confiscated animal parts and products of endangered animal species for the purpose of production of molecular evidence of animal hunting and related crime in the court of law, so that the human violation to the wildlife resources could be controlled.

Still another object is to provide a universal technique to have an idea of the geographical location of the commitment of wildlife crime based on the haplotype of poached animal identified by the universal primer invented.

Another object is to provide a universal technique of animal identification to detect the adulteration of animal meat/products in vegetarian food product for the purpose of food fortification, by the food fortification agencies.

Yet another object is to provide a universal technique for detection of the origin of blood or blood stains etc collected from the scene of crime related to offences such as murder, rape etc, in order to establish the origin of blood found at scene of crime when it sounds as if criminals have wontedly spread the blood of an animal at the scene of crime, to confuse the crime investigation agencies and forensic scientists with human blood.

Another object is to invent and authenticate a universal technique that can be converted to a (a) 'MOLECUALR KIT' and (b) 'DNA CHIPS' based application to meet the requirements of above objectives.

SUMMARY OF THE INVENTION

Accordingly, the invention provides novel universal primers that can amplify the fragment of cytochrome b gene of any animal species in polymerase chain reaction (PCR) and reveal the identity of the biological material of any unknown animal origin

DETAILED DESCRIPTION OF THE INVENTION

Keeping in view the above objectives, the cytochrome b gene sequences (1140 bp) of 221 distantly related animal species (listed in Table 1) representing various families were obtained from public database NCBI (http://www.ncbi.nlm.nih.gov). These sequences were aligned using the software *Clustal X(1.8)*(NCBI, USA) and a fragment (of 472 bp, alignment shown in Table 2) of gene was identified which had all the features mentioned above under column 1, 2 and 3 of sub-heading 'Objectives of invention'. As for the identity of this fragment we would like to mention that it includes the nucleotides between 398 to 869 in *Antilope cervicapra* and *Felis catus*; however, 399 to 870 in *Homo sapiens sapiens* species. Except at few positions (marked as star (*) in Table 2, the nucleotide sequences of this fragment are highly variable amongst the animal species, giving rise to their unique molecular signature. These molecular signatures are characteristic of its species and form the basis of revealing the identity of the biological material of an unknown animal origin by the procedure invented by us. Considering *Antilope cervicapra* as a representative species, the sequence of this fragment is mentioned herewith:

Mitochondrial cytochrome b gene sequence (398-869 bp) of *Antilope cervicapra:*

"taccatgaggacaaatatctttttgaggagcaacagtcatcaccaatctcctttcagcaatcccatacatcggtacaaacctagtaga atgaatctgaggagggttctcagtagataaagcaacccttacccgatttttcgccttccactttatcctcccatttatcattgcagccctta ccatagtacacctactgtttctccacgaaacaggatccaacaacccacaggaatctcatcgacgcagacaaaattccattccaccc ctactacactatcaaagatatcctaggagctctactattaattttaaccctcatgcttctagtcctattctcaccggacctgcttggagacc cagacaactataccagcaaacccacttaatacacccccacatatcaagcccgaatgatacttcctatttgcatacgcaatcctccga tcaattcctaacaaactaggagg"

A pair of universal primer was designed to amplify this fragment in polymerase chain reaction (PCR). These primers were named as 'mcb398' and 'mcb869' because of its property to amplify a region of <u>m</u>itochondrial <u>c</u>ytochrome <u>b</u> gene between nucleotides <u>398</u> to <u>869</u> of *Antilope cervicapra*, a representative animal species for this invention. We took

this animal species as representative species because the idea of developing such a novel primers came in the mind of inventors while they were working on the genome of this animal in Centre for Cellular and Molecular Biology, Hyderabad, India. These primers work universally because its 3' end are highly conserved amongst a vast range of animal species (shown in Table 2). As mentioned above, the DNA fragment (sequence of which is shown above) targeted by these primers is highly polymorphic inter-specifically; however, it is monomorphic among the individual of same species (Tables 6, 7a, 7b, 7c, 7d and 8, respectively). These unique features of the targeted region enable these primers to generate the molecular signatures of an individual species; thereby, enabling them to differentiate amongst the animals of different species (see in Figure 1c). The variation within the fragment amplified by these primers increase with increasing distances of evolutionary lineages of two animals (Table 8). These unique features of the fragment amplified by the universal primers 'mcb398' and 'mcb869' invented by the applicants fulfill the objectives of invention.

Thus, the primers invented by us can generate the molecular signature from any biological material of unknown animal origin, which actually is the characteristic of its family, genus and more precisely, the species. When these signatures are compared *in-silico* with the signatures already available in public databases (viz., GenBank, NCBI database etc) using '*BLAST* software'[73], it indicates identity of the family, genus or species of the analyzed material, which in turn is confirmed practically by comparing with the reference animals of the revealed family, genus or species, by including them in the further analysis by the primers 'mcb398' and 'mcb869'. The complete procedure involved in the *analyses* (the word, '*analyses*' should be understood with the stepwise procedure to establish the identity of the biological remain of any unknown animal origin for the aims mentioned in columns 1-13 under sub-heading 'Objectives of invention') is briefed under 'Examples 5 and 6, respectively, as well as illustrated in Figures 1a, 1b and 1c, respectively.

BRIEF DESCRIPTION OF DRAWING AND TABLES

Figure 1a. Illustration of the step-wise procedure involved in *analyses*. The unknown biological material i.e. 'adil.flesh' refers to the confiscated skin mentioned in 'Example 6'. The arrow marks indicate the stepwise procedure involved. The brief description of Figure 1a is as follows:

The biological material i.e. the confiscated skin 'adil.flesh' was subjected to DNA isolation using the standard procedures[74]. The DNA obtained was amplified using the primers 'mcb398' and 'mcb869' in PCR, fractionated in 2% (w/v) agarose gel, visualized and photographed under UV light using Gel Documentation System (Syngene, USA). The lane 'M' shown in the photograph represents the molecular weight marker (Marker XIII, Boehringer mannheim). Lane 1 shows the PCR amplicon (472 bp) obtained from 'adil.flesh' using primers 'mcb398' and 'mcb869'. The PCR amplicon obtained were sequenced at both the strand using "ABI Prism 3700 DNA Analyzes, PE-Applied Bio-systems). The chromatogram shows the sequences (about 80 bp long, i.e. between 150-230 bp of sequence (328 bp), revealed from the PCR product of 472 bp length) obtained from 'adil.flesh'.

Figure 1b.   Illustrates the further steps involved in *analyses*. The sequence (328 bp) revealed from 'adil.flesh' was subjected to homology search in *nr (i.e. non-redundant)* database of Netional Centre for Biological Information (NCBI), USA. The sequences producing significant alignments are shown along with its bits score and E values. It indicates the extent of homology amongst the sequence enquired (i.e. the 328 bp sequence from adil.flesh) and the sequences registered in *nr* database of NCBI. BLAST analysis revealed the highest homology of the sequence revealed from 'adil.flesh' with the sequence of *Panthera pardus* (gene bank registration number 'AY005809'), indicating the identity of adil.flesh as that of a leopard (*Panthera pardus*) origin. Figure 1b further illustrates the multiple alignments of the sequences obtained from reference animals (listed in Table 5) along with the sequence obtained from 'adil.flesh'. The sequences of 'adil.flesh' is similar to the sequences of 'gz1L' further confirming the identity of the source of confiscated remain 'adil.flesh' as that of a *Panthera pardus* origin.

Figure 1c illustrates the NJ-tree (Neighbor Joining tree) constructed using CLUSTAL X (1.8) from the sequences revealed from 'adil.flesh' and reference animals listed in Table 5. The animals belonging to similar species cluster together; however, the animals of different species group in different clusters. The confiscated material under investigation (i.e. 'adil.flesh') clusters with 'gz1L' (i.e. the known normal leopard *'Panthera pardus'*) indicating the identity of the species of 'adil.flesh' as that of a *Panthera pardus* source.

Figure 2 shows the Agarose gel electrophorogram showing the PCR amplicons (472 bp) obtained from the reference animals of family felidae listed in Table 5, using universal primers 'mcb398 and 'mcb869'. Description of different lanes is as follows:

Lanes 1-21: The PCR profiles of the animals 1-21, respectively, listed in Table 5.

Lane 22: The PCR profiles of DNA isolated from confiscated skin of unknown animal origin 'i.e. adil.flesh'

Lane 23: Negative control (no DNA)

Lane M: Molecular weight marker (marker XIII, Boehringer mannheim)

Figure 3. Shows PCR amplicons obtained from animals listed in Table 9. The primers used in PCR are 'AFF' and 'AFR'. The description of different lanes shown is as follows:

Lane 1-4: The PCR profiles of animals 1-4, respectively, listed in Table 9, showing amplicons of 354 bp.

Lane M: Molecular weight marker (marker XIII, Boehringer mannheim)

Figure 4. Shows PCR amplicons obtained from animals listed in Table 12. This experiment demonstrates the universal nature of our primers among a vast range of animal species. Description of different lanes shown is as follows:

Lanes 1-23: The PCR profiles of the animals 1-23, respectively, listed in Table 12. The PCR product of 472 bp is amplified universally from all the animal species analyzed.

Lane 24: Negative control (no DNA)

Lane M: Molecular weight marker (marker XIII, Boehringer mannheim)

Table 1. List of 221 animal species used for *In-silico* analysis to design the universal primers 'mcb398' and 'mcb869'. Table also demonstrate the 'P,S scores' of 'mcb398' and 'mcb869' for different templates. The descriptions of various symbols used in this table are as follows:

Symbol (#) refers to Number

Symbol (*) refers to the animal species which is either protected species (listed in Wildlife (Protection) Act , 1972 (Central Act NO 53 of 1972), or an endangered/rare animal species

Symbol ($P,S/F) refers to Probability of match and Stability of match of primer 'mcb398' with different templates (i.e. the cytochrome b gene from different species origin). A higher P,S score refers to the higher probabilities of significant amplification of specific template by the primer. It is calculated by *Amplify (1.2)* software.

Symbol (ψP,S/R) refers to Probability of match and Stability of match of primer 'mcb869' with different templates. A higher P,S score refers to the higher probabilities of significant amplification of specific template by the primer. It is calculated by *Amplify (1.2)* software.

Table 2. Multiple sequence alignment of 472 bp fragment of mitochondrial cytochrome b gene (identified by inventors to fulfill the requirements of column 1, 2 and 3 mention under sub-heading 'Objectives of invention') of 221 animal species listed in Table 1. Alignments also show the binding sites for universal primers 'mcb398' and 'mcb869'. The symbol (*) refers to the nucleotide bases which are conserved amongst 221 animal species listed in Table 1). The alignments have been done using software *CLUSTAL X (1.8)*. The nucleitide positions that are unmarked are variable amongst 221 animal species analyzed. These variable sites together constitute the molecular signature of an individual species, giving rise to molecular basis of species identification by our primers.

Table 3. Results of the blast analysis of the sequence revealed from 'adil.flesh' in *'mito'* database of NCBI. It shows the most significant alignment of cytochrome b sequence (328 bp) revealed from confiscated skin piece 'adil.flesh' with *felis catus* cytochrome b gene sequence (genbank registration number NC_001700.1, bits score 365, E value, e-101) registered in NCBI database (bits score 365 and E value e-101). It gives an indication that the species of analyzed material belongs to family felidae. It also fulfills the requirements of column 6 mention above under sub-heading 'Objectives of invention'.

Table 4. Results of the blast analysis of the sequence revealed from 'adil.flesh' in *'nr'* database of NCBI. It shows the most significant alignment of cytochrome b sequence (328 bp) revealed from confiscated skin piece 'adil.flesh' with *Panthera pardus* cytochrome b gene sequence (genbank registration number AY005809, bits score 603, E value, e-170) registered in NCBI database. It gives an indication that the species of analyzed material belongs to *Panthera paurdus* origin. It also fulfills the requirements of column 6 mention above under sub-heading 'Objectives of invention'.

Table 5. Reference animal belonging to family felidae selected for comparison with 'adil.flesh' to confirm the findings of BLAST analysis results of which are mentioned in Table 3 and 4, respectively. The animals listed in SN. 1-21 represent different species of family felidae. SN. 22 and 23 are primate species taken for out-group comparisons.

Table 6 Multiple sequence alignments of cytochrome b sequences (328 bp) revealed from 'adil.flesh' and reference animals listed in Table 5. The positions that have a common nucleotide in all the animal species under investigation are shown with a star (*) mark; however, the positions that are variable in any of the animals under investigation are unmarked. The nucleotides at these positions constitute the molecular signature of an individual species, which are unique and highly specific for its species. These signatures are

the molecular basis of identification of individual animal species using our primers 'mcb398' and 'mcb869'.

Table 7 (Tables 7a, 7b, 7c and 7d). The comparison of the molecular signatures of different animal species investigated along with 'adil.flesh', the confiscated skin of unknown animal origin. This table demonstrates the variable positions (i.e. the positions which are not marked with star (*) symbol in Table 6), amongst the 328 bp fragment revealed from the animals listed in Table 5. The dot (.) mark represents the presence of the similar nucleotide as listed in lane 1 i.e. the sequence from "adil.flesh" at that position. It demonstrates that the signatures of each species are unique and specific to its species. The molecular signatures of 'adil.flesh' are comparable (except for position 37 which has a transition from 'T' to 'C') to the molecular signature of 'gz1L' i.e. the known leopard *'Panthera pardus'* source, indicating the identity of the source of confiscated skin 'adil.flesh' as that of a leopard *'Panthera pardus'* source. The nucleotide variations (at the positions 153, 198, 223, 264, among the known leopards, (i.e. gz1L, gz2L, and gz3L, respectively)), give an idea about the geographical habitat of each animals. Various studies referring to molecular evolution of different animal species support this hypothesis[75]; however, it could further be confirmed by taking the reference animals from different geographical areas and analyzing by our primers 'mcb 398' and 'mcb869'. If we could generate the database of different haplotypes (i.e. habitat specific molecular signatures) of the animal species, it would also enable our primers to reveal the geographical location of the commitment of wildlife crime.

Table 8. Percent similarity matrix calculated by pair-vise comparisons of nucleotide sequences aligned (illustrated in Table 6). The cytochrome b gene sequence of DNA isolated from confiscated material had maximum similarity (99.7% and 98.2%, with the lineages of animals 'gz2L' and 'gz3L', respectively) with the sequences obtained from known normal leopard source, indicating its identity as that of a leopard origin. The similarity matrix has been calculated using the software *PHYLIP (3.5)*.

Table 9. Animals selected for validation of minimum P,S score for efficient amplification of cytochrome b gene of different origin by the primers 'mcb398' and 'mcb869'. P,S score of primers 'AFF' and 'AFR' for these animals are shown.

Table 10. BLAST analysis of primers 'mcb398' in *nr* database of NCBI . It demonstrates that the 3' end of this primer is highly conserved among a vast range of animal species. It also shows the significant homology among the primer and templates (i.e. the cytochrome b gene fragment of different animal species), confirming the universal nature of our primer

Table 11. BLAST analysis of primers 'mcb869' in *nr* database of NCBI. It demonstrates that the 3' end of this primer is highly conserved among a vast range of animal species. It also shows the significant homology among the primer and templates (i.e. the cytochrome b gene fragment of different animal species), confirming the universal nature of our primer.

Table 12. Other animal belonging to distantly related animal species, investigated to confirm the universal nature of primers 'mcb398' and 'mcb869'. Gel photograph showing the PCR amplicons from these animals are shown in Figure 4.

The mitochondrial cytochrome b gene has very widely been used in molecular taxonomic studies. It has immense capabilities to reveal different evolutionary lineages of animals in family, genus and species specific manner. It has also been used to classify the population of a particular species according to its demographic distributions[75]. The vast database of cytochrome b sequences of different animal species has accumulated in public databases such as Genbank and NCBI[1-65]. We have explored these unique characteristics of cytochrome b gene to establish the identity of confiscated remains of any unknown animal by inventing a pair of novel primers, 'mcb398' and 'mcb869', that can amplify a small fragment (472 bp) of cytochrome b gene of wide range of animal species in universal manner. These primers work universally because its 3' ends target within a highly conserved region.

The fragment of cytochrome b gene identified had all the features mentioned in columns 1, 2 and 3 listed under sub-heading 'Objective of invention'. We identified this fragment by aligning the cytochrome b gene sequences (1140 bp) of 221 different animal species listed in Table 1. These sequences are publicly available in NCBI DNA databases. These sequences were aligned using the software *CLUSTAL X (1.8)*. As mentioned before, the 472 bp fragment of cytochrome b gene identified by us to have the features mentioned in columns 1, 2 and 3 listed under sub-heading 'Objective of invention' includes the nucleotides between 398 to 869 in *Antilope cervicapra* and *Felis catus*; however, 399 to 870 in *Homo sapiens sapiens* species. Except at few positions (marked as star (*) in Table 2, the nucleotide sequences of this fragment are highly variable amongst the animal species, revealing the identity of the biological material belonging to that of an unknown animal origin by the procedure invented by us. As for identity of this fragment we are considering *Antilope cervicapra* as a representative species, and the sequence the above fragment of cytochrome b gene of *Antilope cervicapra* is mentioned herewith:

Mitochondrial cytochrome b gene sequence (398-869 bp) of *Antilope cervicapra*

"taccatgaggacaaatatcttttttgaggagcaacagtcatcaccaatctcctttcagcaatcccatacatcggtacaaacctagtaga
atgaatctgaggagggttctcagtagataaagcaaccctttacccgatttttcgccttccactttatcctcccatttatcattgcagcccta
ccatagtacacctactgtttctccacgaaacaggatccaacaacccacaggaatctcatcagacgcagacaaaattccattccaccc
ctactacactatcaaagatatcctaggagctctactattaattttaaccctcatgcttctagtcctattctcaccggacctgcttggagacc
cagacaactataccagcaaacccacttaatacacccccacatatcaagcccgaatgatacttcctatttgcatacgcaatcctccga
tcaattcctaacaaactaggagg

Table 2 presents the alignment of the above fragment of cytochrome b gene of 221 animal species. Each species in table 2 has been represented by a unique code, which is decoded in Table 1. We selected these species to represent the vast range of animal families of distant orders. Of 221 species, about 65 were the protected/endangered or rare species listed in Wildlife (Protection) Act , 1972 (Central Act NO 53 of 1972). These species are marked with symbol (*) in Table 1. The NCBI accession number refers to its registration number in NCBI database and the number in superscript represent the reference cited. Based on the aligned cytochrome b sequences of different 221 animal species the primers designed were as follow:

Primers name	Sequence (5'-3')
'mcb398'	"TACCATGAGGACAAATATCATTCTG"
'mcb869'	"CCTCCTAGTTTGTTAGGGATTGATCG"

Tables 2, 10 and 11, respectively, demonstrates that the 3' ends of the primers are highly conserved amongst all the animal species analyzed *in-silico* (In total 221 animal species listed in Table 1 and about 500 species listed in Tables 10 and 11, respectively) Also, the 5' end of the primers were selected within the conserved region of cytochrome b gene to improve the probability and stability of match of the primers to their target sequences (i.e. the above mentioned 472 bp fragment of cytochrome b gene). The primers were thoroughly checked for internal stabilities, loop or dimmer formation using different software viz., 'Amplify (1.2)', 'Primer3' (http://www.genome.wi.mit.edu/cgi-bin/primer/primer3.cgi) as well as manually. . We assigned the P,S score (P=Probability of match, S=Stability of match) to the primers for each template using the software *Amplify (1.2)*. The higher scores of P and S ensure a good amplification if all other conditions standard (which are mentioned under 'Example 3') are optimum. The Highest score for 'mcb398' was 98,63 (i.e. the situation where the primer has perfect match with template); however, the highest P, S for 'mcb869' was recorded as 98, 68 for a complete match between the primer and template.

The lowest P,S score observed for 'mcb398' was 81,50 for species *Talpa europaea* whereas 'mcb869' had a high P, S score for this species (92, 57). The another species which have lowest P, S score for one of the two primers were *Eumeces egregious* and *Equus ainus*. *Eumeces egregious* had P, S score 86, 55 and 73,51 for 'mcb398' and 'mcb869', respectively; however, the P, S score of *Equus ainus was calculated as* 91,61 and 73, 51 for 'mcb398' and 'mcb869', respectively. All other animals had higher P, S scores then the above mentioned species. To ensure that these primers would work efficiently with the DNA template from the animals having the lowest P, S score for one of the primers, we designed an another experiment to validate the lower limits of one of the two primers sufficient for efficient amplification in PCR. We designed an another primer pair (AFF= 5'tagtagaatgaatctgaggagg3' and AFR=5'atgcaaataggaagtatcattc3'.) having more mis-pairing at their annealing sites (but not at ends), therefore have less internal stability and lower P, S scores for its templates (listed in Table 9). The P,S scores of 'AFF' and 'AFR' were as calculated as low as 41 and 49 for *Platanista gangetica* and *Sus scrofa* These species were amplified efficiently using the primers 'AFF' and 'AFR' (results shown in Figure 3) (keeping all other conditions standard i.e. the conditions mentioned in 'Example 3'). The lowest P,S scores (86, 55 and 73,51 for species *Eumeces egregious*) for our primers 'mcb398' and 'mcb869', respectively, were higher then the above range of combined P, S scores of 'AFF' and 'AFR' for species *Sus scrofa* (87, 52 and 87, 41), which was efficiently amplified by the primers 'AFF' and 'AFR'. It gives an indication that the primers 'mcb 398' and 'mcb 869' would work with all the species including *Eumeces egregious* efficiently to give rise to the expected product in PCR. This experiment confirmed that the primers 'mcb398' and 'mcb 869' are capable of amplifying the cytochrome b fragment of most of the animal species in a universal manner.
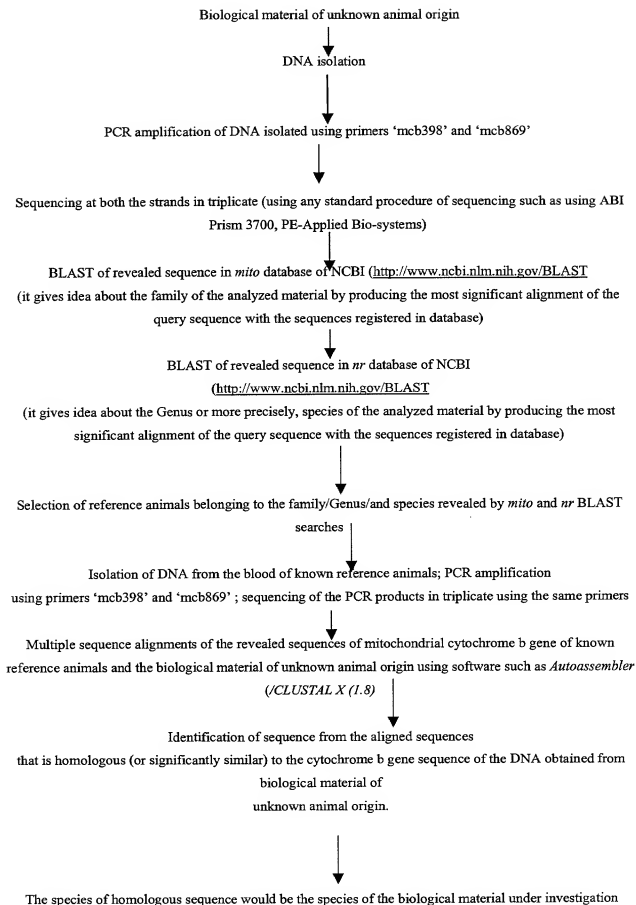
For further confirmation of universal nature of our primers, we blasted the sequence of our primers against the *mito* and *nr* databases of NCBI using BLAST software. The results of these analyses are shown in Tables 10, and 11, respectively.

Finally, the universal nature of the primers was tested in our laboratory with some more animal species listed in Table 12. These primers amplified all the animal species efficiently, giving rise to the band of expected size (472 bp). The results are shown in Figure 4. This experiments substantiated the results of P,S analysis and other *in-silico* analyses to show that the primers 'mcb398'and 'mcb 869' are universal primers.

The flow chart of establishing identity of the species of biological material of unknown animal origin using primers 'mcb398' and 'mcb869'

Biological material of unknown animal origin

↓

DNA isolation

↓

PCR amplification of DNA isolated using primers 'mcb398' and 'mcb869'

↓

Sequencing at both the strands in triplicate (using any standard procedure of sequencing such as using ABI Prism 3700, PE-Applied Bio-systems)

↓

BLAST of revealed sequence in *mito* database of NCBI (http://www.ncbi.nlm.nih.gov/BLAST (it gives idea about the family of the analyzed material by producing the most significant alignment of the query sequence with the sequences registered in database)

↓

BLAST of revealed sequence in *nr* database of NCBI
(http://www.ncbi.nlm.nih.gov/BLAST
(it gives idea about the Genus or more precisely, species of the analyzed material by producing the most significant alignment of the query sequence with the sequences registered in database)

↓

Selection of reference animals belonging to the family/Genus/and species revealed by *mito* and *nr* BLAST searches

↓

Isolation of DNA from the blood of known reference animals; PCR amplification using primers 'mcb398' and 'mcb869' ; sequencing of the PCR products in triplicate using the same primers

↓

Multiple sequence alignments of the revealed sequences of mitochondrial cytochrome b gene of known reference animals and the biological material of unknown animal origin using software such as *Autoassembler (/CLUSTAL X (1.8)*

↓

Identification of sequence from the aligned sequences that is homologous (or significantly similar) to the cytochrome b gene sequence of the DNA obtained from biological material of unknown animal origin.

↓

The species of homologous sequence would be the species of the biological material under investigation

Examples

Example 1.

Example for identification of a fragment of cytochrome b gene fulfilling the requirements of columns 1, 2 and 3 mentioned under sub-heading 'Objectives of invention' of heading 'Brief summary of invention'

The cytochrome b molecule has very vastly been used in molecular taxonomic studies. Being a slow evolving gene, It has a tremendous information in its nucleotide sequences to distinguish the animals to their family, genus and species sources[1-65]. A vast database of the sequences of cytochrome b gene of different animal species has accumulated in the *nr* and *mito* databases of NCBI. We have explored these qualities of cytochrome b gene to establish the identity of confiscated remains of unknown animal origin to its family, genus and species sources. For this purpose, we have identified a fragment of cytochrome b gene which is highly polymorphic inter-specifically, however, it is monomorphic among the individual of same species, therefore it can group the individual of an unknown species with the known individuals of reference species to which it belongs. In order to amplify this fragment from DNA isolated form any unknown origin, it was necessary that it remain flanked with the highly conserved sequences amongst a vast range of animal families. To identify such a unique fragment within the cytochrome b gene, we aligned the sequences of 221 distantly related animal species (listed in Table 1) representing various families using software CLUSTAL X (1.8). These sequences were obtained from public database NCBI (http://www.ncbi.nlm.nih.gov). The aligned data was examined carefully for the conserved sites amongst all the species included in *in-silico* analysis. We identified a fragment (472 bp) of cytochrome b gene that was fulfilling all the requirements mentioned above and also under column 1, 2 and 3 of sub-heading 'Objectives of invention'.

As for the identity of this fragment we would like to mention that it includes the nucleotides between 398 to 869 in *Antilope cervicapra* and *Felis catus*; however, 399 to 870 in *Homo sapiens sapiens* species. Except at few positions marked as star (*) in Table 2, the nucleotide sequences of this fragment are highly variable amongst the animal species, giving rise to their unique molecular signature. These molecular signatures are characteristic of its species and form the basis of revealing the identity of the biological material of an

unknown animal origin by the procedure invented by us. Considering *Antilope cervicapra* as a representative species, the sequence of this fragment is mentioned herewith:

Mitochondrial cytochrome b gene sequence (398-869 bp) of *Antilope cervicapra*

"taccatgaggacaaatatcttttgaggagcaacagtcatcaccaatctcctttcagcaatcccatacatcggtacaaacctagtaga atgaatctgaggaggggttctcagtagataaagcaaccettacccgattttcgccttccactttatcctcccatttatcattgcagcccta ccatagtacacctactgtttctccacgaaacaggatccaacaacccacaggaatctcatcagacgcagacaaaattccattccaccc ctactacacatcaaagatatcctaggagctctactattaattttaaccctcatgcttctagtcctattctcaccggacctgcttggagacc cagacaactatacaccagcaaacccacttaatacaccccacatatcaagcccgaatgatacttcctatttgcatacgcaatcctccga tcaattcctaacaaactaggagg"

Example 2:

Example for development of universal primers to amplify the fragment identified mentioned under 'Example 1'
A pair of universal primer was designed which has the following features:

1. It targets the fragment identified (mentioned under 'Example 1') to amplify it in polymerase chain reaction (PCR).

2. Its 3' and 5' ends that are highly conserved (marked as star (*) in Table 2), amongst a vast range of animal species ensuring the amplification of the fragment mentioned above in a universal manner. The sequencing of the fragment amplified by these primes reveals the molecular signature of the species of analyzed material, which on comparison with the sequences of the known reference animals reveals the identity of the species of unknown biological material under investigation.

3. The tm (melting temperature) of both primers was almost similar (about 58 degree centigrade) ensuring the significant annealing of both the primers to its template, therefore significant amplification of targeted region in PCR.

4. The internal stability and P, S, score of the primers were ensured higher while designing it. The possibilities of internal loop formation, dimmer formation etc were also excluded by selecting its sequence uniquely. This ensured that the primer would

be a good primer to be used in PCR for amplification of DNA from unknown animal origin.

5. The 3' end of the primers were ensured to have either 'G' or 'C' to increase the probability of strong bonding at its 3'ends, which is necessary for efficient amplification of DNA template in PCR. It also strengthens the universal nature of the primer.

6. The sequences of the primers were ensured to be unique so that it does not give rise to non-specific and spurious products in PCR leading to confusion. It improved the efficiency and quality of the technique invented by us.

7. These primers were named as 'mcb398' and 'mcb869' because of its property to amplify a region of mitochondrial cytochrome b gene between nucleotides 398 to 869 of *Antilope cervicapra*, a representative animal species for this invention. We took this animal species as representative species because the idea of developing such a novel primers came in the mind of inventors while they were working on the genome of this animal in Centre for Cellular and Molecular Biology, Hyderabad, India.

8. The sequences of the universal primers invented are as follows:

Primers name   Sequence (5'-3')

'mcb398'   "TACCATGAGGACAAATATCATTCTG"

'mcb869'   "CCTCCTAGTTTGTTAGGGATTGATCG"

Example 3:

Example for development of universal PCR conditions to ensure the amplification of a template of any unknown origin in PCR, hence strengthening the universal nature of the technique invented by us

The PCR conditions developed had the following unique features:

1 These were capable of amplifying the DNA template of any animal origin in an universal manner using the universal primers mentioned under 'Example 2'.

2. The conditions were selected to ensure the comparable annealing temperature for both the primers i.e. 'mcb398' and 'mcb869'.

3. The PCR conditions standardized herewith are universal; therefore, the possibility of PCR failure with a template of unknown origin due to non-standard conditions is

excluded. It ensures the universal nature of our technique to be used in wildlife forensics.

4. The universal conditions mentioned above are:

Amplification reactions should be carried out in 20 μl reaction volume containing approximately 20 ηg of template DNA, 100μm each of dNTPs, 1.25 pmole of each primer, 1.5mM MgCl$_2$, 0.5 unit of Ampli*Taq* Gold (Perkin-Elmer-Cetus, USA) DNA polymerase and 1X PCR buffer (10mM Tris-HCl, pH 8.3, and 50mM KCl). The amplification profiles followed should be: an initial denaturation at 95^{0}C for 10 min, followed by 35 cycles each of denaturation at 95^{0}C for 45 s, annealing at 51^{0}C for 1 min, and extension at 72^{0}C for 2 min. The extension step at 35th cycles should be held for 10 min.

Example 4:

Establishing the universal nature of our primer and experimental evidences to demonstrate the universal nature of primers:

The universal nature of the primers 'mcb398' and 'mcb 869' was ensured by the following measures:

(a) Selecting the primers from the aligned cytochrome b gene sequences of 221 animal of distantly related species:

The cytochrome b gene sequences (1140 bp) were aligned using software *CLUSTAL X (1.8)*. The region of cytochrome b gene that was most conserved amongst 221 animal species was selected to design the primers.

(b) Selecting the 3' and 5' ends of the primers at the highly conserved positions of cytochrome b gene:

The 3' and 5' ends of the primers were ensured to anneal to a highly conserved position amongst 221 animal species representing a vast range of animal families. It was done to ensure an efficient amplification of all the species in PCR. These positions are shown with star (*) mark in Table 2.

(c) Ensuring either 'G' or 'C' at the 3' end of the primers:

It was ensured the primers to have either 'G' or 'C' at its 3' ends as these are the nucleotides that ensure the strong bonding at the 3' ends of the primers due to three hydrogen bonds while pairing with each other. The strong bonding at 3' ends helps the primers to anneal properly with its template resulting in significant amplification in PCR.

(d) Selecting the sequences of the primers to ensure a higher internal stability, higher P, S score, and no primer dimmer and loop formation:

The sequences of the primers were selected to have a high P, S score for a vast range of animal species (Shown in Table 1). The care was taken to exclude the possibilities of loop or primer dimmer formation that could reduce the efficiency of the primers in PCR.

(e) Selecting the sequence of the primers with a comparable melting temperature:

The sequences of the primers were selected to have a comparable melting temperature so that these could work together to amplify a DNA template in PCR at a similar annealing temperature. The melting temperature of both the primers was about 58 degree centigrade and the annealing temperature used in PCR is 51 degree centigrade.

Experimental evidences to demonstrate the universal nature of primers:

(1) Evidence from *In-silico* analysis :

(a) Selecting the primers within the most conserved region of mitochondrial cytochrome b gene

As mentioned above, the primers were designed to anneal within a highly conserved region of mitochondrial cytochrome b gene fragment of 472 bp. Table 2 presents the alignment of the above fragment of cytochrome b gene of 221 animal species representing a vast range of animal families. The conserved positions of nucleotide sequences are shown with star (*) mark in Table 2

Table 2 also demonstrates that the 3' ends of the primers are highly conserved amongst all the animal species analyzed *in-silico*. In the aligned sequences, the conserved nucleotides are marked with symbol (*). Also, the 5' end of the primers were selected within the conserved region of cytochrome b gene to improve the probability and stability of match of the primers to their target sequences (i.e. the above mentioned 472 bp fragment of cytochrome b gene). The primers were thoroughly checked for internal stabilities, loop or dimmer formation using different software viz., *'Amplify (1.2)'*, *'Primer3'* (http://www.genome.wi.mit.edu/cgi-bin/primer/primer3.cgi) as well as manually.

(b) P, S, score analysis:

We assigned the P,S score (P=Probability of match, S=Stability of match) to the primers for each template using the software *Amplify (1.2)*. The higher scores of P and S ensure a good amplification if all other conditions standard (which are mentioned under 'Example 3') are optimum. The Highest score for 'mcb398' was 98,63 (i.e. the situation

where the primer has perfect match with template); however, the highest P, S for 'mcb869' was recorded as 98, 68 for a complete match between the primer and template. The lowest P,S score observed for 'mcb398' was 81,50 for species *Talpa europaea* whereas 'mcb869' had a high P, S score for this species (92, 57). The another species which have lowest P, S score for one of the two primers were *Eumeces egregious* and *Equus ainus*. *Eumeces egregious* had P, S score 86, 55 and 73,51 for 'mcb398' and 'mcb869', respectively; however, the P, S score of *Equus ainus was calculated as* 91,61 and 73, 51 for 'mcb398' and 'mcb869', respectively. All other animals had higher P, S scores then the above mentioned species. To ensure that these primers would work efficiently with the DNA template from the animals having the lowest P, S score for one of the primers, we designed an another experiment to validate the lower limits of one of the two primers sufficient for efficient amplification in PCR. We designed an another primer pair (AFF= $^{5'}$ctagtagaatgaatctgaggagg$^{3'}$ and AFR= $^{5'}$tatgcaaataggaagtatcattc$^{3'}$.) that have more mis-pairing at their annealing sites (but not at ends), therefore have less internal stability and lower P, S scores for its templates (listed in Table 9). The P,S scores of 'AFF' and 'AFR' were as calculated as low as 41 and 49 for *Platanista gangetica* and *Sus scrofa* These species were amplified efficiently using the primers 'AFF' and 'AFR' (results shown in Figure 3) (keeping all other conditions standard i.e. the conditions mentioned in 'Example 3'). The lowest P,S scores (86, 55 and 73,51 for species *Eumeces egregious*) for our primers 'mcb398' and 'mcb869', respectively, were higher then the above range of combined P, S scores of 'AFF' and 'AFR' for species *Sus scrofa* (87, 52 and 87, 41), which was efficiently amplified by the primers 'AFF' and 'AFR'. It gives an indication that the primers 'mcb 398' and 'mcb 869' would work with all the species including *Eumeces egregious* efficiently to give rise to the expected product in PCR. This experiment confirmed that the primers 'mcb398' and 'mcb 869' are capable of amplifying the cytochrome b fragment of most of the animal species in a universal manner.

© BLAST analysis:

The sequences of primers 'mcb398' and 'mcb869' were blasted against mito and nr databases of NCBI to see its significant alignments with the sequences registered in GenBank. As expected, the most significant alignments of the sequences were found with the cytochrome b gene regions (within the 472 bp fragment mentioned in 'Example 1') of different animal species. This analysis also showed that the 3' as well as 5' ends of the primers were highly conserved amongst a vast range of animal species, confirming the universal nature of the primers (Tables 10 and 11, respectively)
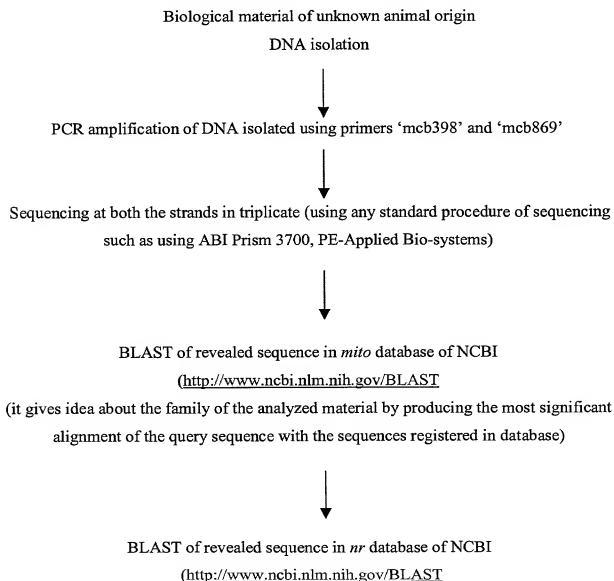
(2) Evidence from bench work/experiments done in laboratory conditions:

The DNA from different animals belonging to distantly related species (mentioned in Table 12) was isolated and subjected to PCR amplification using the primers invented by us i.e. the primers 'mcb398' and 'mcb869' The PCR products amplified were resolved in agarose gel by electrophoresis and visualized under UV light. The PCR products of expected size (472bp) were obtained from all the animals confirming the universal nature of our primers. These results are shown in Figure 4.

Example 5:

Example to establish the identity of confiscated remains from unknown animal origin using the universal primers 'mcb398' and 'mcb869'.

The step-vise procedure to establish the identity of the biological material from an unknown animal source is mentioned below:

<div align="center">

Biological material of unknown animal origin

DNA isolation

↓

PCR amplification of DNA isolated using primers 'mcb398' and 'mcb869'

↓

Sequencing at both the strands in triplicate (using any standard procedure of sequencing such as using ABI Prism 3700, PE-Applied Bio-systems)

↓

BLAST of revealed sequence in *mito* database of NCBI
(http://www.ncbi.nlm.nih.gov/BLAST

(it gives idea about the family of the analyzed material by producing the most significant alignment of the query sequence with the sequences registered in database)

↓

BLAST of revealed sequence in *nr* database of NCBI
(http://www.ncbi.nlm.nih.gov/BLAST

</div>

(it gives idea about the Genus or more precisely, species of the analyzed material by producing the most significant alignment of the query sequence with the sequences registered in database)

↓

Selection of reference animals belonging to the family/Genus/and species revealed by *mito* and *nr* BLAST searches

↓

Isolation of DNA from the blood of known reference animals; PCR amplification using primers 'mcb398' and 'mcb869' ; sequencing of the PCR products in triplicate using the same primers

↓

Multiple sequence alignments of the revealed sequences of mitochondrial cytochrome b gene of known reference animals and the biological material of unknown animal origin using software such as *Autoassembler/CLUSTAL X (1.8)*

↓

Identification of sequence from the aligned sequences that is homologous (or significantly similar) to the cytochrome b gene sequence of the DNA obtained from biological material of unknown animal origin.

↓

The species of homologous sequence would be the species of the biological material under investigation

↓

Application of the above information for the objectives mentioned in columns 7-13 under sub-heading 'Objective of invention' of heading 'Summary of invention'

Example 6:

The actual execution of the technique invented

As a first application and to demonstrate the ease and utility of this method, we investigated a case of forensic identification submitted at our laboratory to seek scientific opinion on animal hunting evidence. In this case, we received the half burned remains of an unknown animal, confiscated by the crime investigation agencies. The DNA was isolated from the above material following standard methods[74] and subjected to PCR amplification using the primers mentioned above (viz., 'mcb398' and 'mcb869'). Amplification reactions were carried out in 20 μl reaction volume containing 20 ηg of template DNA, 100μm each of dNTPs, 1.5mM MgCl$_2$, 0.5 unit of Ampli*Taq* Gold (Perkin-Elmer-Cetus, USA) DNA polymerase and 1X PCR buffer (10mM Tris-HCl, pH 8.3, and 50mM KCl). The amplification profiles followed were: an initial denaturation at 95^{0}C for 10 min, followed by 35 cycles each of denaturation at 95^{0}C for 45 s, annealing at 51^{0}C for 1 min, and extension at 72^{0}C for 2 min. The extension step at 35th cycles was held for 10 min.

The PCR products obtained were sequenced in automated work station (ABI Prism 3700, PE-Biosystems) on both strands in triplicate and the sequence resolved (328 bp, shown in Figure 1a) was blasted against *mito* databases of NCBI using BLAST program[73]. The most significant alignment (bits Value 365, E value e^{-101}) of this sequence was produced with the cytochrome b gene sequence of *Felis catus*, (Table 3) indicating that species of analyzed material belongs to family felidae. Further, the above sequence revealed from the confiscated remain was blasted against *nr* databases of NCBI using BLAST program. The most significant alignment (bits Value 603, E value e^{-170}) of this sequence was produced with the cytochrome b gene sequence of *Panthera pardus* (Table 4), indicating the identity of the analyzed material as that of a *Panthera pardus* source. Based on this information, we selected the reference animals listed in Table 5 representing different species and subspecies of felidae. The DNA isolated from reference animals was amplified and sequenced on both strands in triplicate using the primer pair mentioned above. Consensus sequences obtained were aligned using program *CLUSTAL X (1.8)* (Table 6). Sequence comparisons identified 113 variable sites in total amongst all animals analyzed (Table 7). Pair-vise comparisons of sequences were performed to find out the variation among different animals investigated. All the species investigated were differentiated by a their unique nucleotides sequences. The molecular signatures of different reference animals

were compared with the molecular signature of the confiscated skin 'adil.flesh'. Table 7 demonstrate that the maximum similarity of the adil.flesh with 'gz1l' i.e. known Leopard (*Panthera pardus*) species, indicating the identity of the adil.flesh, the confiscated skin, as that of a Panthera pardus origin. We also calculated the similarity matrix showing the pair-vise similarity amongst the animal species under investigation using *PHYLIP* software This matrix is shown in Table 8. It demonstrates that the animals belonging to different species had more variation; however, the animals of same species had maximum similarity among their cytochrome b sequences. The cytochrome b gene sequence of DNA isolated from confiscated material had maximum similarity with the sequences obtained from known Leopard source(99.7%, and 98.2 with 'gz1l' and 'gz2l', respectively); establishing the identity of the source of confiscated material as that of a Normal leopard (*Penthera pardus*) species. The step-vise procedure involved in above analysis is illustrated in Figure 1a, 1b and 1c, respectively.

Thus, the primers invented by us can generate the molecular signature from any biological material of unknown animal origin, which actually is the characteristic of its family, genus and more precisely, the species. When these signatures are compared *in-silico* with the signatures already available in public databases (viz., GenBank, NCBI database etc) using *BLAST* software[73], it indicates identity of the family, genus or species of the analyzed material, which in turn is confirmed practically by comparing with the reference animals of the revealed family, genus or species, by including them in the further analysis by the primers 'mcb398' and 'mcb869'. Application of the information revealed could be in fulfilling the requirements of objectives mentioned in columns 7-13 under sub-heading 'Objective of invention' of heading 'Summary of invention'

The method of the invention can be used to establish the identity of confiscated animal parts and products is one of the key requirements of wildlife identification in forensics. It is needed to establish the crime with the criminal beyond a reasonable doubt to avoid the human violation of wildlife resources. Various morphological biochemical and molecular approaches have been given for this purpose; however, none of the current methods is universally applicable to detect the mutilated animal remains of unknown origin. We have identified a fragment on the mitochondrial cytochrome b gene, which has enormous information to differentiate among various animal species back to the family, genus and species sources. We have also found that this fragment is flanked by the highly conserved sequences amongst a vast range of animal species. We invented a pair of universal primer

that can amplify this fragment of DNA isolated from the biological material of an unknown animal origin in polymerase chain reaction (PCR) to reveal its identity at species and sub-species sources. This novel invention has great potential to revolutionize the whole scenario of wildlife forensic identification and crime investigation.

Table 1. List of 221 animal species used for *In-silico* analysis to design the universal primers 'mcb398' and 'mcb869'. Table also demonstrate the 'P,S scores' of 'mcb398' and 'mcb869' for different templates. The descriptions of various symbols used in this table are as follows:

Symbol (#) refers to <u>N</u>umber

Symbol (*) refers to the animal species which is either protected species (listed in Wildlife (Protection) Act , 1972 (Central Act NO 53 of 1972), or an endangered/rare animal species

Symbol ($^\delta$P,S/F) refers to <u>P</u>robability of match and <u>S</u>tability of match of primer 'mcb398' with different templates (i.e. the cytochrome b gene from different species origin). A higher P,S score refers to the higher probabilities of significant amplification of specific template by the primer. It is calculated by *Amplify (1.2)* software.

Symbol ($^\psi$P,S/R) refers to <u>P</u>robability of match and <u>S</u>tability of match of primer 'mcb869' with different templates. A higher P,S score refers to the higher probabilities of significant amplification of specific template by the primer. It is calculated by *Amplify (1.2)* software.

Table 1. The animal species included in the study for *in-silico* analysis

SN.	Code	Name	NCBI accession #	$P,S/F	ᵚP,S/R
1	aep.mel	*Aepyceros melampus*	AF036289[1]	97, 60	94, 62
2	ore.ore	*Oreotragus oreotragus*	AF036288[1]	88, 52	94, 62
3	add.nas	*Addax nasomaculatus*	AF034722[2]	97, 60	95, 66
4	ory.dam	*Oryx damah*	AJ222685[1]	90, 58	95, 66
5	hip.equ	*Hippotragus equinus*	AF022060[3]	98, 63	85, 55
6	alc.bus	*Alcelaphus buselaphus*	AJ222681[1]	97, 60	98, 68
7	sig.lic	*Sigmoceros lichtensteini*	AF034967[4]	97, 60	98, 68
8	bea.hun	*Beatragus hunteri*	AF034968[4]	97, 60	94, 62
9	dam.lun	*Damaliscus lunatus*	AF016635[3]	97, 60	77, 55
10	con.tau	*Connochaetes taurinus*	AF016638[3]	82, 56	93, 62
11	bis.bon	*Bison bonasus*	Y15005[5]	90, 58	87, 63
12	bos.gru	*Bos grunniens**	AF091631[6]	90, 58	94, 62
13	bos.tra	*Bos tragocamelus**	AJ222679[1]	90, 58	95, 66
14	buba.bub	*Bubalus bubalis**	D34637[7]	97, 60	93, 64
15	bub.min	*Bubalus mindorensis*	D82895[8]	97, 60	87, 62
16	tra.ang	*Tragelaphus angasii*	AF091633[6]	97, 60	87, 63
17	tra.eur	*Tragelaphus eurycerus*	AF036276[1]	90, 58	97, 64
18	nem.cau	*Nemorhaedus caudatus**	U17861[9]	95, 61	93, 59
19	pse.nay	*Pseudois nayaur*	AF034732[2]	89, 55	89, 59
20	amm.ler	*Ammotragus lervia*	AF034731[2]	94, 58	97, 63
21	cap.fal	*Capra falconeri**	D84202[10]	98, 63	95, 66
22	cap.ibe	*Capra ibex**	AF034735[2]	98, 63	98, 58
23	hem.jem	*Hemitragus jemlahicus**	AF034733[2]	95, 61	90, 61
24	rup.pyr	*Rupicapra pyrenaica*	AF034726[2]	95, 61	89, 59
25	rup.rup	*Rupicapra rupicapra*	AF034725[2]	95, 61	94, 64
26	pan.hod	*Pantholops hodgsoni*	AF034724[2]	98, 63	95, 66
27	bud.tax.tax	*Budorcas taxicolor taxicolor**	U17868[9]	90, 58	95, 66
28	ovi.amm	*Ovis ammon**	AF034727[2]	98, 63	97, 64
29	ovi.vig	*Ovis vignei**	AF034729[2]	98, 63	97, 64
30	cap.cri	*Capcornis crispus**	AJ304502[11]	98, 63	94, 63
31	ovi.mos	*Ovibos moschatus*	U17862[9]	98, 63	92, 61
32	ore.ame	*Oreamnos americanus*	AF190632[12]	98, 63	94, 62
33	cep.dor	*Cephalophus dorsalis*	AF091634[6]	97, 58	90, 61
34	cep.max	*Cephalophus maxwellii*	AF096629[13]	97, 60	88, 53
35	alc.alc	*Alces alces*	AJ000026[14]	95, 61	93, 59
36	hyd.ine	*Hydropotes inermis*	AJ000028[14]	97, 60	90, 63
37	mun.mun	*Muntiacus muntjak**	AF042718[15]	90, 58	93, 64
38	cer.ele.kan	*Cervus elaphus kansuensis**	AB021098[16]	98, 63	82, 59
39	cer.ele.xan	*Cervus elaphus xanthopygus**	AB021097[16]	98, 63	82, 59
40	cer.ele.can	*Cervus elaphus canadensis**	AB021096[16]	98, 63	90, 61
41	cer.nip.ce	*Cervus nippon centralis*	AB021094[16]	98, 63	90, 61
42	cer.nip.ye	*Cervus nippon yesoensis*	AB021095[16]	98, 63	90, 61
43	cer.nip.ke	*Cervus nippon keramae*	AB021091[16]	98, 63	90, 61
44	cer.nip.pu	*Cervus nippon pulchellus*	AB021090[16]	98, 63	90, 61
45	cer.nip.ni	*Cervus nippon nippon*	AB021093[16]	98, 63	90, 61
46	cer.ela.sc	*Cervus elaphus scoticus*	AB021099[16]	98, 63	90, 61

47	cer.dam	*Cervus dama*	AJ000022[14]	98, 63	88, 53
48	ran.tar	*Rangifer tarandus*	AJ000029[14]	98, 63	89, 57
49	mos.fus	*Moschus fuscus**	AF026888[17]	90, 59	90, 61
50	mos.leu	*Moschus leucogaster**	AF026889[17]	90, 59	90, 61
51	mos.chr	*Moschus chrysogaster**	AF026887[17]	90, 59	90, 61
52	mos.ber	*Moschus berezovskii**	AF026886[17]	90, 59	90, 61
53	mos.mos	*Moschus moschiferus**	AF026883[17]	90, 59	92, 61
54	kob.ell	*Kobus ellipsiprymnus*	AF022059[3]	91, 61	95, 66
55	kob.meg	*Kobus megaceros*	AJ222686[1]	91, 61	83, 56
56	red.aru	*Redunca arundinum*	AF096628[13]	91, 61	94, 62
57	red.ful	*Redunca fulvorufula*	AF036284[1]	89, 57	94, 62
58	neo.mos	*Neotragus moschatus*	AJ222683[1]	89, 57	94, 62
59	pel.cap	*Pelea capreolus*	AF022055[3]	91, 61	90, 61
60	ant.cer	*Antilope cervicapra**	AF022058[3]	82, 56	93, 64
61	sai.tat	*Saiga tatarica*	AF064487[18]	91, 61	92, 61
62	gaz.dam	*Gazella dama*	AF025954[3]	91, 61	92, 61
63	our.our	*Ourebia ourebi*	AF036288[1]	82, 56	82, 59
64	gaz.gaz	*Gazella gazella**	AJ222682[1]	91, 61	89, 57
65	rap.mel	*Raphicerus melanotis*	AF022053[3]	81, 54	80, 50
66	mad.kir	*Madoqua kirkii*	AF022070[3]	90, 58	97, 65
67	ant.ame	*Antilocapra americana*	AF091629[6]	98, 63	98, 68
68	tra.jav	*Tragulus javanicus**	D32189[19]	86, 57	86, 59
69	tra.nap	*Tragulus napu**	X56288[20]	81, 52	93, 58
70	bal.acu	*Balaenoptera acutorostrata*	X75753[21]	89, 56	97, 61
71	bal.bon	*Balaenoptera bonaensis*	X75581[21]	89, 56	93, 59
72	bal.bor	*Balaenoptera borealis**	X75582[21]	89, 56	93, 59
73	bal.edi	*Balaenoptera edeni*	X75583[21]	89, 56	88, 54
74	esc.rob	*Eschrichtius robustus**	X75585[21]	97, 61	86, 57
75	bal.mus	*Balaenoptera musculus**	NC_001601[22]	97, 57	93, 59
76	meg.nov	*Megaptera novaeangliae**	X75584[21]	97, 61	94, 63
77	bal.phy	*Balaenoptera physalus**	NC_001321[23]	97, 57	94, 63
78	cap.mar	*Caperea marginata*	X75586[21]	93, 55	91, 53
79	cep.com	*Cephalorhynchus commersonii*	AF084073[24]	85, 51	88, 55
80	cep.eut	*Cephalorhynchus eutropia**	AF084072[24]	85, 51	92, 59
81	lag.obl	*Lagenorhynchus obliquidens*	AF084067[24]	94, 59	92, 59
82	cep.hea	*Cephalorhynchus heavisidii*	AF084070[24]	89, 56	97, 63
83	cep.hec	*cephalorhynchus hectori**	AF084071[24]	89, 56	92, 59
84	lag.aus	*Lagenorhynchus australis*	AF084069[24]	86, 54	92, 59
85	lag.cru	*Lagenorhynchus cruciger*	AF084068[24]	86, 54	92, 59
86	lag.obs	*Lagenorhynchus obscurus*	AF084066[24]	86, 54	92, 59
87	lis.bor	*Lissodelphis borealis*	AF084064[24]	85, 51	92, 59
88	lis.per	*Lissodelphis peronii*	AF084065[24]	86, 54	92, 59
89	glo.mac	*Globicephala macrorhynchus*	AF084055[24]	94, 59	88, 55
90	glo.mel	*Globicephala melas*	AF084056[24]	94, 59	88, 55
91	fer.att	*Feresa attenuata**	AF084052[24]	94, 59	92, 59
92	pep.ele	*Peponocephala electra**	AF084053[24]	94, 59	88, 55
93	gra.gri	*Grampus griseus*	AF084059[24]	97, 61	89, 59
94	pse.cra	*Pseudorca crassidens**	AF084057[24]	94, 59	92, 59
95	lag.acu	*Lagenorhynchus acutus*	AF084075[24]	98, 63	89, 59
96	orci.bre	*Orcinus orca*	AF084061[24]	86, 57	82, 52

97	orca.bre	*Orcaella brevirostris*	AF084063[24]	86, 57	91, 54
98	del.cap	*Delphinus capensis*	AF084087[24]	96, 54	97, 63
99	del.tro	*Delphinus tropicalis*	AF084088[24]	97, 57	97, 63
100	del.del	*Delphinus delphis*	AF084085[24]	97, 57	97, 63
101	sten.cly	*Stenella clymene*	AF084083[24]	97, 57	97, 63
102	sten.coe	*Stenella coeruleoalba*	AF084082[24]	97, 57	97, 66
103	tur.adu	*Tursiops aduncus*	AF084092[24]	97, 57	97, 63
104	sten.fro	*Stenella frontalis*	AF084090[24]	97, 57	97, 63
105	saus.chi	*Sousa chinensis*	AF084080[24]	97, 57	88, 59
106	sten.lon	*Stenella longirostris*	AF084103[24]	97, 61	97, 63
107	turs.tru	*Tursiops truncatus*	AF084095[24]	97, 57	96, 59
108	lage.alb	*Lagenorhynchus alborostris*	AF084074[24]	97, 61	97, 66
109	sten.bre	*Steno bredanensis*	AF084077[24]	97, 61	94, 64
110	sota.flu	*Sotalia fluviatilis*	AF304067[25]	97, 61	97, 63
111	del.leu	*Delphinapterus leucas*	U72037[26]	97, 61	95, 66
112	mono.mon	*Monodon monoceros*	U72038[26]	97, 61	95, 66
113	plat.gan	*Platanista gangetica* *	AF304070[25]	97, 61	86, 59
114	plat.min	*Platanista minor* *	X92541[27]	97, 61	86, 59
115	kogi.bre	*Kogia breviceps*	U72040[26]	97, 59	90, 63
116	kogi.sim	*Kogia simus*	AF304072[28]	96, 55	92, 63
117	phys.cat	*Physeter catodon*	AF304073[25]	97, 57	80, 58
118	lipo.vex	*Lipotes vexillifer* *	AF304071[25]	89, 56	88, 53
119	phoc.sin	*phocoena sinus*	AF084051[24]	87, 49	92, 62
120	bera.bai	*Berardius bairdii*	X92541[27]	96, 55	90, 59
121	ziph.car	*Ziphius cavirostris*	X92540[27]	97, 61	89, 57
122	meso.eur	*Mesoplodon europaeus*	X92537[27]	97, 57	90, 61
123	meso.bid	*Mesoplodon bidens*	X92538[27]	97, 61	92, 61
124	meso.den	*Mesoplodon densirostris*	X92536[27]	91, 61	94, 63
125	hype.amp	*Hyperoodon ampullatus* *	X92539[27]	97, 61	90, 65
126	meso.per	*Mesoplodon peruvianus*	AF304074[28]	97, 61	86, 58
127	pont.bla	*Pontoporia blainvillei*	AF304069[25]	92, 59	88, 55
128	hipp.amp	*Hippopotamus amphibius*	Y08813[29]	92, 58	95, 66
129	hex.lib	*Hexaprotodon liberiensis*	Y08814[29]	98, 63	97, 66
130	rhin.son	*Rhinoceros sondaicus* *	AJ245725[30]	90, 59	87, 61
131	cera	*Ceratotherium simum*	NC_001808[32]	90, 59	90, 63
132	dic.sum	*Dicerorhinus sumatrensis*	AJ245723[30]	90, 59	86, 57
133	equu	*Equus asinus*	NC_001788[31]	91, 61	73, 51
134	baby.bab	*Babyrousa babyrussa*	Z50106[33]	89, 56	85, 56
135	phac.afr	*Phacochoerus africanus*	Z50090[33]	90, 59	87, 54
136	sus.scr.ew	*Sus scrofa haplotype EWB3* *	AF136549[34]	97, 57	83, 54
137	sus.bar	*Sus barbatus*	Z50107[33]	97, 57	85, 55
138	lama.gla	*Lama glama*	U06429[35]	89, 55	85, 53
139	lama.gua	*lama guanicoe*	Y08812[29]	88, 54	86, 57
140	vic.vic	*Vicugna vicugna*	U06430[35]	89, 55	85, 53
141	cam.bac	*Camelus bactrianus*	U06427[35]	94, 58	86, 58
142	arc.for	*Arctocephalus forsteri*	X82293[36]	97, 60	87, 64
143	arc.gaz	*Arctocephalus gazella*	X82292[36]	94, 58	87, 64
144	eum.jub	*Eumetopias jubatus*	X82311[36]	97, 57	86, 57
145	zal.cal	*Zalophus californianus*	X82310[36]	89, 55	86, 57
146	odo.ros	*Odobenus rosmarus*	X82299[36]	91, 61	81, 52

147	pho.vit	*Phoca vitulina*	X82306[36]	90, 58	87, 64
148	pho.fascia	*Phoca fasciata*	X82302[36]	98, 63	95, 66
149	pho.gro	*Phoca groenlandica*	X82303[36]	92, 59	90, 61
150	cys.cri	*Cystophora cristata*	X82294[36]	89, 56	87, 64
151	hyd.lep	*Hydrurga leptonyx*	X82297[36]	89, 55	82, 54
152	lep.wed	*Leptonychotes weddelli*	X72005[37]	98, 63	91, 66
153	mir.leo	*Mirounga leonina*	X82298[36]	89, 55	82, 59
154	eri.bar	*Erignathus barbatus*	X82295[36]	89, 56	87, 63
155	mon.sch	*Monachus schauinslandi*	X72209[37]	91, 61	87, 60
156	hela.mal	*Helarctos malayanus* *	U18899[38]	84, 54	90, 63
157	sel.thi	*Selenarctos thibetanus* *	AB020910[39]	89, 57	87, 64
158	ail.ful	*Ailurus fulgen* *s	X94919[40]	93, 55	87, 64
159	fel	*Felis catus*	NC_001700[41]	85, 56	90, 63
160	can	*Canis familiaris*	NC_002008[42]	98, 58	84, 54
161	tal	*Talpa europaea*	NC_002391[43]	81, 50	92, 57
162	gla.sab	*Glaucomys sabrinus*	AF011738[44]	90, 59	82, 54
163	gla.vol	*Glaucomys volans*	AB030261[45]	90, 59	87, 60
164	hyl.pha	*Hylopetes phayrei* *	AB030263[45]	91, 61	81, 50
165	pet.set	*Petinomys setosus* *	AB030260[45]	91, 61	81, 50
166	bel.pea	*Belomys pearsonii* *	AB030262[45]	91, 61	87, 64
167	pte.mom	*Pteromys momonga* *	AB030263[45]	97, 61	90, 63
168	gala.demi	*Galagoides demidoff*	AF271411[46]	97, 58	87, 64
169	pero.pot	*Perodicticus potto*	AF271413[46]	97, 60	87, 63
170	gala.mat	*Galago matschiei*	AF271409[46]	97, 60	90, 61
171	gala.moh	*Galago moholi*	AF271410[46]	97, 57	95, 66
172	oto.gar	*Otolemur garnettii*	AF271412[46]	92, 58	87, 60
173	lor.tar	*Loris tardigradus* *	U53581[47]	97, 60	93, 59
174	nyc.cou	*Nycticebus coucang* *	U53580[47]	97, 60	95, 66
175	mus	*Mus musculus*	NC_001569[48]	97, 60	86, 59
176	gorr	*Gorilla gorilla*	NC_001645[49]	89, 57	80, 58
177	homo	*Homo sapiens sapiens*	NC_001807[50]	96, 55	84, 64
178	dug.dug	*Dugong dugon* *	U07564[51]	97, 60	89, 59
179	ele.max	*Elephas maximus* *	AB002412[52]	97, 60	76, 57
180	afr.con	*Afropavo congensis*	AF013760[53]	97, 58	87, 63
181	pavo.mut	*Pavo muticus* *	AF013763[53]	97, 57	87, 63
182	tra.bly	*Tragopan blythii* *	AF200722[54]	89, 55	85, 57
183	tra.sat	*Tragopan satyra* *	AF229837[54]	89, 55	86, 61
184	tra.cob	*Tragopan caboti*	AF200723[54]	89, 55	86, 61
185	tra.tem	*Tragopan temminckii* *	AF028802[55]	89, 55	81, 56
186	arg.arg	*Argusianus argus*	AF013761[53]	89, 55	87, 63
187	cat.wal	*Catreus wallichi* *	AF028792[53]	88, 54	85, 57
188	cro.cro	*Crossoptilon crossoptilon* *	AF028794[53]	89, 55	85, 57
189	sym.ree	*Symmaticus reevesi* *	AF028801[53]	89, 55	85, 57
190	bam.tho	*Bambusicola thoracica* *	AF028790[53]	80, 48	94, 64
191	fra.fra	*Francolinus francolinus*	AF013762[53]	97, 58	86, 61
192	ith.cru	*Ithaginis cruentus* *	AF068193[53]	98, 63	85, 57
193	ant.par	*Anthropoides paradisea*	U27557[56]	85, 56	82, 58
194	ant.vir	*Anthropoides virgo*	U27545[56]	84, 54	82, 52
195	gru.ant.an	*Grus antigone antigone*	U11060[57]	90, 58	87, 63
196	gru.ant.gi	*Grus antigone gillae*	U11064[57]	90, 58	87, 63

197	gru.any.sh	*Grus antigone sharpei*	U11061[57]	90, 58	87, 63
198	gru.leu	*Grus leucogeranus* *	U27549[56]	90, 58	87, 63
199	gru.can.pr	*Grus canadensis pratensis*	U27553[56]	97, 60	87, 63
200	gru.can.ro	*Grus canadensis rowani*	U27552[56]	97, 60	87, 63
201	gru.can.ta	*Grus canadensis tabida*	U27551[56]	98, 63	87, 63
202	gru.can.ca	*Grus canadensis canadensis*	U27554[56]	97, 61	87, 63
203	gru.ame	*Grus americana*	U27555[56]	90, 58	87, 63
204	gru.gru	*Grus grus*	U27546[56]	89, 54	87, 63
205	gru.mon	*Grus monacha* *	U27548[56]	90, 58	87, 63
206	gru.nig	*Grus nigricollis* *	U27547[56]	90, 58	87, 63
207	gru.jap	*Grus japonensis*	U27550[56]	81, 54	87, 63
208	cic.boy	*Ciconia boyciana* *	NC_002196[58]	94, 58	79, 60
209	rhe.ame	*Rhea americana*	AF090339[59]	93, 63	79, 60
210	ant.alb	*Anthracoceros albirostris* *	U89190[60]	97, 61	86, 59
211	fal.fam	*Falco femoralis*	U83310[61]	97, 61	86, 60
212	fal.ver	*Falco verpertinus*	U83311[61]	97, 61	85, 57
213	fal.par	*Falco peregrinus* *	U83307[61]	97, 61	84, 52
214	fal.spa	*Falco sparverius*	U83306[61]	92, 59	80, 51
215	ayt.ame	*Aythya americana*	NC_000877[62]	98, 63	94, 62
216	smi.sha	*Smithornis sharpei*	NC_000879[59]	97, 58	90, 61
217	vid.cha	*Vidua chalybeata*	NC_000880[59]	97, 60	87, 64
218	chry.pic	*Chrysemys picta*	NC_002073[63]	89, 56	86, 57
219	emy.orb.ku	*Emys orbicularis*	AJ131425[64]	90, 59	94, 63
220	che.mud	*Chelonia mydas* *	AB012104[65]	90, 58	94, 63
221	eum.egr	*Eumeces egregius*	AB016606[65]	86, 55	73, 51

Table 2. Multiple sequence alignment of 472 bp fragment of mitochondrial cytochrome b gene (identified by inventors to fulfill the requirements of column 1, 2 and 3 mention under sub-heading 'Objectives of invention') of 221 animal species listed in Table 1. Alignments also show the binding sites for universal primers 'mcb398' and 'mcb869'. The symbol (*) refers to the nucleotide bases which are conserved amongst 221 animal species listed in Table 1). The alignments have been done using software *CLUSTAL X (1.8)*. The nucleitide positions that are unmarked are variable amongst 221 animal species analyzed. These variable sites together constitute the molecular signature of an individual species, giving rise to molecular basis of species identification by our primers.

Table 2. Multiple sequence alignment of 472 bp fregment of mitochondrial
cytochrome b gene of 221 animal species

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PRIMER 'mcb398'    TACCATGAGGACAAATATCATTCTG
                   * ** ** ** ** ** ** ** ** *   *  ** ** ** ** **   *
** **
aep.mel
TGCCATGAGGACAAATATCATTCTGAGGAGCAACAGTCATTACAAATCTCCTCTCAGCAA 60
ore.ore
TTCCGTGAGGACAAATATCATTTTGAGGGGCTACAGTCATTACTAATCTCCTCTCAGCAA 60
add.nas
TGCCATGAGGACAAATATCATTCTGAGGAGCAACAGTCATCACCAACCTTCTCTCAGCAA 60
ory.dam
TACCATGAGGACAAATATCATTTTGAGGGGCAACAGTTATCACTAACCTTCTCTCAGCAA 60
hip.equ
TACCATGAGGACAAATATCATTCTGAGGAGCAACAGTCATCACCAACCTCCTCTCAGCAA 60
alc.bus
TGCCATGAGGACAAATATCATTCTGAGGGGCAACAGTCATCACCAATCTCCTCTCAGCAA 60
sig.lic
TGCCATGAGGACAAATATCATTCTGAGGGGCAACAGTCATCACCAATCTCCTCTCAGCAA 60
bea.hun
TGCCATGAGGACAAATATCATTCTGAGGAGCAACAGTCATCACCAACCTCCTCTCAGCAA 60
dam.lun
TGCCATGAGGACAAATATCATTCTGAGGAGCAACAGTCATCACTAACCTCCTCTCAGCAA 60
con.tau
TACCATGAGGACAAATATCCTTTTGAGGAGCAACAGTCATCACCAACCTCCTCTCAGCAA 60
amm.ler
TGCCATGAGGACAGATATCATTCTGAGGGGCAACAGTCATCACCAACCTTCTCTCAGCAA 60
pse.nay
TGCCATGAGGACAAATATCATTTTGAGGGGCAACAGTCATCACCAACCTTCTCTCAGCAA 60
cap.ibe
TACCATGAGGACAAATATCATTCTGAGGGGCAACAGTCATCACTAACCTTCTCTCAGCAA 60
hem.jem
TACCATGAGGACAGATATCATTCTGAGGGGCAACAGTCATCACCAACCTTCTCTCAGCAA 60
cap.fal
TACCATGAGGACAAATATCATTCTGAGGGGCAACAGTCATCACCAATCTCCTCTCAGCAA 60
rup.pyr
TACCATGAGGACAGATATCATTCTGAGGAGCAACAGTTATTACCAATCTCCTCTCAGCAA 60
rup.rup
TACCATGAGGACAGATATCATTCTGGGGAGCAACAGTTATTACCAACCTCCTCTCAGCGA 60
nem.cau
TACCATGAGGACAGATATCATTCTGAGGGGCAACAGTTATTACCAATCTTCTCTCAGCAA 60
bud.tax.tax
TACCATGAGGACAAATATCATTTTGAGGAGCAACAGTCATTACCAACCTCCTCTCAGCAA 60
pan.hod
TACCATGAGGACAAATATCATTCTGAGGAGCAACAGTAATTACCAACCTCCTTTCAGCAA 60
ovi.amm
TACCATGAGGACAAATATCATTCTGAGGAGCAACAGTTATTACCAACCTCCTTTCAGCAA 60
ovi.vig
TACCATGAGGACAAATATCATTCTGAGGAGCAACAGTTATTACCAACCTCCTTTCAGCAA 60
cap.cri
TACCATGAGGACAAATATCATTCTGAGGGGCTACAGTCATTACTAACCTCCTCTCAGCAA 60
ovi.mos
TACCATGAGGACAAATATCATTCTGAGGAGCTACAGTCATCACTAACCTCCTCTCAGCAA 60
ore.ame
TACCATGAGGACAAATATCATTCTGAGGAGCAACAGTCATNACCAATCTCCTTTCAGCAA 60
cep.dor
TCCCATGAGGGCAAATATCATTCTGAGGAGCCACAGTCATTACCAACCTCCTCTCAGCAA 60
cep.max
TCCCATGAGGACAAATATCATTCTGAGGAGCCACAGTCATTACCAACCTCCTCTCAGCAA 60
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bis.bon
TACCATGAGGACAAATATCATTTTGAGGAGCAACAGTCATTACCAACCTCCTATCAGCAA 60
bos.gru
TACCATGAGGACAAATATCATTTTGAGGGGCAACAGTCATTACCAACCTCCTATCAGCAA 60
bos.tra
TACCATGAGGACAAATATCATTTTGAGGAGCAACAGTTATTACCAATCTATTATCAGCAA 60
bub.min
TGCCATGAGGACAAATATCATTCTGAGGAGCAACAGTCATCACCAACCTTCTCTCAGCAA 60
buba.bub
TGCCATGAGGACAAATATCATTCTGAGGGGCAACAGTCATCACCAACCTTCTCTCAGCAA 60
tra.ang
TGCCATGAGGACAAATATCATTCTGAGGAGCAACGGTCATCACAAACCTCCTATCAGCAA 60
tra.eur
TACCATGAGGACAAATATCATTTTGAGGAGCAACAGTCATCACAAACCTTCTATCAGCAA 60
kob.ell
TACCATGAGGACAAATATCCTTCTGAGGAGCAACAGTCATCACCAATCTCCTTTCAGCAA 60
kob.meg
TACCATGAGGACAAATATCCTTCTGAGGAGCGACAGTCATCACTAATCTCCTTTCAGCAA 60
red.aru
TACCATGAGGACAAATATCCTTCTGAGGAGCAACAGTTATCACTAATCTTCTCTCAGCAA 60
red.ful
TGCCATGGGGACAAATATCCTTCTGAGGAGCAACAGTTATCACTAACCTTCTCTCAGCAA 60
neo.mos
TGCCATGGGGACAAATATCCTTCTGAGGAGCAACAGTCATCACCAATCTACTATCAGCAA 60
pel.cap
TACCATGAGGACAAATATCCTTCTGAGGAGCAACAGTCATCACCAACCTTCTCTCAGCAA 60
gaz.dam
TACCATGAGGACAAATATCTTTCTGAGGGGCAACAGTTATCACTAACCTCCTCTCAGCAA 60
our.our
TACCATGAGGACAAATATCCTTTTGAGGAGCAACAGTCATCACCAACCTCCTCTCAGCAA 60
ant.cer
TACCATGAGGACAAATATCTTTTTGAGGAGCAACAGTCATCACCAATCTCCTTTCAGCAA 60
sai.tat
TACCATGAGGACAAATATCTTTCTGAGGAGCAACAGTCATCACCAATCTCCTTTCAGCAA 60
mad.kir
TGCCATGAGGACAAATATCCTTCTGAGGAGCAACAGTTATCACTAACCTCCTCTCAGCAA 60
rap.mel
TACCATGGGGACAAATATCCTTTTGAGGAGCAACAGTCATCACTAATCTCCTCTCAGCAA 60
gaz.gaz
TACCATGAGGACAAATATCTTTCTGAGGAGCAACAGTTATCACGAACCTCCTCTCAGCAA 60
ant.ame
TACCATGAGGACAAATATCATTCTGAGGGGCAACAGTCATTACTAACCTACTCTCAGCAA 60
hyd.ine
TTCCATGAGGACAAATATCATTCTGAGGAGCAACGGTCATTACTAATCTCCTGTCAGCAA 60
mun.mun
TACCATGAGGACAAATATCATTTTGAGGAGCAACAGTCATCACTAACCTCCTTTCAGCAA 60
alc.alc
TACCATGAGGACAGATATCATTCTGAGGGGCAACAGTCATTACTAACCTCCTTTCAGCAA 60
cer.ela.kan
TACCATGAGGACAAATATCATTCTGAGGAGCAACAGTCATTACCAACCTTCTCTCAGCAA 60
cer.ela.xan
TACCATGAGGACAAATATCATTCTGAGGAGCAACGGTCATTACCAACCTTCTCTCAGCAA 60
cer.ela.can
TACCATGAGGACAAATATCATTCTGAGGAGCAACAGTCATTACCAACCTTCTCTCAGCAA 60
cer.nip.cent
TACCATGAGGACAAATATCATTCTGAGGAGCAACAGTCATTACCAACCTCCTCTCAGCAA 60
cer.nip.yes
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cer.nip.ker
TACCATGAGGACAAATATCATTCTGAGGAGCAACAGTCATTACCAACCTTCTCTCAGCAA 60
cer.nip.pul
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TACCATGAGGACAAATATCATTCTGAGGAGCAACAGTCATTACCAACCTTCTCTCAGCAA 60
cer.nip.nip
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cer.ela.sco
TACCATGAGGACAAATATCATTCTGAGGAGCAACAGTCATCACCAACCTTCTCTCAGCAA 60
cer.dam
TACCATGAGGACAAATATCATTCTGAGGAGCAACAGTTATTACCAATCTTCTCTCAGCAA 60
ran.tar
TACCATGAGGACAAATATCATTCTGAGGAGCAACAGTTATCACAAACCTCCTCTCAGCAA 60
mos.fus
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mos.leu
TACCTTGAGGACAAATATCTTTCTGAGGAGCAACAGTTATTACCAATCTTCTCTCAGCAA 60
mos.chr
TACCTTGAGGACAAATATCTTTCTGAGGAGCAACAGTTATTACCAATCTTCTCTCAGCAA 60
mos.ber
TACCTTGAGGACAAATATCTTTCTGAGGAGCAACAGTTATTACCAATCTTCTCTCAGCAA 60
mos.mos
TACCTTGAGGACAAATATCTTTCTGAGGAGCAACAGTCATCACTAACCTTCTCTCAGCAA 60
tra.jav
TACCCTGAGGACAGATATCTTTCTGAGGAGCCACAGTCATCACCAACCTCTTATCAGCTA 60
trag.nap
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bala.acu
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bala.bon
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bala.bor
TACCCTGAGGACAAATATCATTTTGAGGCGCAACCGTCATCACCAACCTCTTATCAGCAA 60
bala.edi
TACCCTGAGGACAAATATCATTTTGAGGCGCAACCGTCATCACCAACCTCTTATCAGCAA 60
esch.rob
TACCCTGAGGACAAATATCATTCTGAGGCGCAACCGTTATCACCAACCTCCTATCAGCAA 60
bala.mus
TGCCCTGAGGACAAATATCATTCTGAGGCGCAACCGTCATCACCAACCTCCTATCAGCAA 60
mega.nov
TACCCTGAGGACAAATATCATTCTGAGGCGCAACCGTCATCACCAACCTTCTATCAGCAA 60
bala.phy
TGCCCTGAGGACAAATATCATTCTGAGGCGCAACTGTAATCACTAACCTCCTATCAGCAA 60
cap.mar
TGCCCTGAGGACAGATATCATTCTGAGGCGCAACCGTCATCACCAACCTCCTATCAGCAA 60
ceph.com
TACCCTGGGGACAGATATCATTTTGAGGTGCAACAGTCATCACCAACCTCCTATCAGCAA 60
ceph.eut
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lage.obl
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ceph.hea
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ceph.hec
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lage.aus
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lage.cru
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lage.obs
TACCCTGAGGACAGATATCATTTTGAGGTGCAACAGTCATCACCAACCTCCTATCAGCAA 60
lisso.bor
TACCCTGAGGGCAGATATCATTTTGAGGTGCAACCGTCATCACCAACCTCCTATCAGCAA 60
lisso.per
TACCCTGAGGACAGATATCATTTTGAGGTGCAACCGTCATCACCAACCTCCTATCAGCAA 60
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glo.mac
TACCCTGAGGACAGATATCATTCTGAGGCGCAACCGTCATCACCAATCTCCTATCAGCAA 60
glo.mel
TACCCTGAGGACAGATATCATTCTGAGGCGCAACCGTCATCACCAATCTCCTATCAGCAA 60
fere.att
TACCCTGAGGACAGATATCATTCTGAGGCGCAACCGTCATCACCAATCTCCTATCAGCAA 60
pepo.ele
TACCCTGAGGACAGATATCATTCTGAGGCGCAACCGTCATCACCAATCTCCTATCAGCAA 60
gram.gri
TACCCTGAGGACAAATATCATTCTGAGGCGCAACCGTCATCACCAATCTCCTATCAGCAA 60
pse.cra
TACCCTGAGGACAAATATCATTCTGAGGCGCAACCGTCATCACCAATCTTCTATCAGCAA 60
lage.acu
TACCATGAGGACAAATATCATTCTGAGGCGCAACCGTTATCACCAATCTCCTATCAGCAA 60
orci.bre
TACCCTGAGGACAGATATCTTTCTGAGGCGCAACCGTCATTACTAATCTCCTATCAGCAA 60
orca.bre
TACCCTGAGGACAGATATCCTTCTGAGGTGCAACCGTCATCACCAATCTCCTATCAGCAA 60
del.cap
TGCCCTGGGGACAAATATCATTCTGAGGCGCAACCGTCATCACCAACCTCTTATCAGCAA 60
del.tro
TGCCCTGAGGACAAATATCATTCTGAGGCGCAACCGTCATCACCAACCTCTTATCAGCAA 60
del.del
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lama.gua        ACTTCCTATTTGCATATGCCATCCTACGATCCATCCCCAACAAATTAGGCGG
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cic.boy     ACTTCCTCTTTGCATACGCCATCCTACGCTCCATCCCCAACAAACTAGGAGG
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PRIMER 'mcb869'                     CGATCAATCCCTAACAAACTAGGAGG
                 *  **   *   *     **   *   *    ** **   * ** **      * ** **
```

Table 3. Results of the blast analysis of the sequence revealed from 'adil.flesh' in '*mito*' database of NCBI. It shows the most significant alignment of cytochrome b sequence (328 bp) revealed from confiscated skin piece 'adil.flesh' with *felis catus* cytochrome b gene sequence (genbank registration number NC_001700.1, bits score 365, E value, e-101) registered in NCBI database (bits score 365 and E value e-101). It gives an indication that the species of analyzed material belongs to family felidae. It also fulfills the requirements of column 6 mention above under sub-heading 'Objectives of invention'.

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<b>BLASTN 2.1.2 [Nov-13-2000]</b>


<b><a href="http://www.ncbi.nlm.nih.gov/htbin-
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Altschul, Stephen F., Thomas L. Madden, Alejandro A. Sch&auml;ffer,
Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997),
"Gapped BLAST and PSI-BLAST: a new generation of protein database search
programs",  Nucleic Acids Res. 25:3389-3402.
<p>
RID: 984593689-1224-27770
<p>
<b>Query=</b>
         (328 letters)


<p>
<b>Database:</b> Sequences from complete mitochondrial genomes
          129 sequences; 3,164,247 total letters

<p> <p>If you have any problems or questions with the results of this search
<br>please refer to the <b><a
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Sequences producing significant alignments:                (bits)   Value

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  Database: Sequences from complete mitochondrial genomes
    Posted date:  Jun 28, 2000 10:56 AM
  Number of letters in database: 3,164,247
  Number of sequences in database:  129


Lambda     K        H
    1.37     0.711      1.31

Gapped
Lambda     K        H
    1.37     0.711      1.31


Matrix: blastn matrix:1 -3
Gap Penalties: Existence: 5, Extension: 2
Number of Hits to DB: 788
Number of Sequences: 129
Number of extensions: 788
Number of successful extensions: 168
Number of sequences better than 10.0: 77
length of query: 328
length of database: 3,164,247
effective HSP length: 15
effective length of query: 313
effective length of database: 3,162,312
effective search space: 989803656
effective search space used: 989803656
T: 0
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X1: 6 (11.9 bits)
X2: 15 (29.7 bits)
S1: 12 (24.3 bits)
S2: 14 (28.2 bits)

</PRE>

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Table 4. Results of the blast analysis of the sequence revealed from 'adil.flesh' in 'nr' database of NCBI. It shows the most significant alignment of cytochrome b sequence (328 bp) revealed from confiscated skin piece 'adil.flesh' with *Panthera pardus* cytochrome b gene sequence (genbank registration number AY005809, bits score 603, E value, e-170) registered in NCBI database. It gives an indication that the species of analyzed material belongs to *Panthera paurdus* origin. It also fulfills the requirements of column 6 mention above under sub-heading 'Objectives of invention'.

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<b><a href="http://www.ncbi.nlm.nih.gov/htbin-
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Altschul, Stephen F., Thomas L. Madden, Alejandro A. Sch&auml;ffer,
Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997),
"Gapped BLAST and PSI-BLAST: a new generation of protein database search
programs",  Nucleic Acids Res. 25:3389-3402.
<p>
RID: 984593400-28182-3122
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<b>Query=</b>
        (328 letters)

<p>
<b>Database:</b> nt
        807,597 sequences; 2,863,827,885 total letters

<p> <p>If you have any problems or questions with the results of this search
<br>please refer to the <b><a
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Gapped
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Table 5. Reference animal belonging to family felidae selected for comparison with 'adil.flesh' to confirm the findings of BLAST analysis, results of which are mentioned in Table 3 and 4, respectively. The animals listed in SN. 1-21 represent different species of family felidae. SN. 22 and 23 are primate species taken for out-group comparisons. The samples started with the code 'bhz' were collected from Bhuvaneshwar zoo, the samples with code numbers 'gz' from Guwahati zoo, samples coded with the number 'darz' from Darjeeling zoo, and the samples coded as 'sbz' were collected from Sakkarbaug zoo, India.

Table 5. Reference animals and the allocated code numbers included in the study

SN.	Code number	Name of the animal	Zoological name
1	bhz25t	Indian tiger	*Panthera tigris tigris*
2	bhz26t	Indian tiger	*Panthera tigris tigris*
3	bhz30t	Indian tiger	*Panthera tigris tigris*
4	bhz45t	Indian tiger	*Panthera tigris tigris*
5	bhz56t	Indian tiger	*Panthera tigris tigris*
6	bhz63t	Indian tiger	*Panthera tigris tigris*
7	bhz20wt	Indian white tiger	*Panthera tigris bengalensis*
8	bhz22wt	Indian white tiger	*Panthera tigris bengalensis*
9	bhz23wt	Indian white tiger	*Panthera tigris bengalensis*
10	bhz28wt	Indian white tiger	*Panthera tigris bengalensis*
11	gz1l	Normal leopard	*Panthera pardus*
12	gz2l	Normal leopard	*Panthera pardus*
13	gz3l	Normal leopard	*Panthera pardus*
14	gz21cl	Clouded leopard	*Neofelis nebulosa*
15	gz22cl	Clouded leopard	*Neofelis nebulosa*
16	darz14sl	Snow leopard	*Panthera unicia*
17	darz15sl	Snow leopard	*Panthera unicia*
18	darz16sl	Snow leopard	*Panthera unicia*
19	sbz22al	Asiatic lion	*Panthera leo persica*
20	sbz38al	Asiatic lion	*Panthera leo persica*
21	sbz39al	Asiatic lion	*Panthera leo persica*
22	humsk	Human	*Homo sapiens sapiens*
23	chimss	Chimpanzee	*Pan troglodytes*

Table 6.   **Multiple sequence alignments of cytochrome b sequences (328 bp) revealed from 'adil.flesh' and reference animals listed in Table 5.** The positions that have a common nucleotide in all the animal species under investigation are shown with a star (*) mark; however, the positions that are variable in any of the animals under investigation are unmarked. The nucleotides at these positions constitute the molecular signature of an individual species, which are unique and highly specific for its species. These signatures are the molecular basis of identification of individual animal species using our primers **'mcb398'** and **'mcb869'**.

Table 6. Multiple sequence alignments of the cytochrome b sequences of reference
animals with the sequence obtained from confiscated animal remain

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sbz39al      TGAATCTGAGGAGGCTTCTCAGTAGACAAAGCCACCCTGACACGATTCTTTGCCTTCCAC 60
adil.flesh   TGAATCTGAGGAGGCTTCTCAGTAGACAAAGCTACCCTGACACGATTCTTTGCCTTCCAC 60
gz1nl        TGAATCTGAGGAGGCTTCTCAGTAGACAAAGCTACCTTGACACGATTCTTTGCCTTCCAC 60
gz2nl        TGAATCTGAGGAGGCTTCTCAGTAGACAAAGCTACCTTGACACGATTCTTTGCCTTCCAC 60
gz3nl        TGAATCTGAGGAGGCTTCTCAGTAGACAAAGCTACCTTGACACGATTCTTTGCCTTCCAC 60
bhz23wt      TGAATCTGAGGAGGCTTCTCAGTAGACAAAGCCACCCTGACACGATTCTTTGCCTTCCAC 60
bhz28wt      TGAATCTGAGGAGGCTTCTCAGTAGACAAAGCCACCCTGACACGATTCTTTGCCTTCCAC 60
bhz22wt      TGAATCTGAGGAGGCTTCTCAGTAGACAAAGCCACCCTGACACGATTCTTTGCCTTCCAC 60
bhz20wt      TGAATCTGAGGAGGCTTCTCAGTAGACAAAGCCACCCTGACACGATTCTTTGCCTTCCAC 60
bhz63t       TGAATCTGAGGAGGCTTCTCAGTAGACAAAGCCACCCTGACACGATTCTTTGCCTTCCAC 60
bhz56t       TGAATCTGAGGAGGCTTCTCAGTAGACAAAGCCACCCTGACACGATTCTTTGCCTTCCAC 60
bhz26t       TGAATCTGAGGAGGCTTCTCAGTAGACAAAGCCACCCTGACACGATTCTTTGCCTTCCAC 60
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chimss       TGAATCTGAGGAGGCTACTCAGTAGACAGCCCTACCCTTACACGATTCTTCACCTTCCAC 60
humsk        TGAATCTGAGGAGGCTACTCAGTAGACAGTCCCACCCTCACACGATTCTTTACCTTTCAC 60
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sbz38al      TTCATCCTTCCATTTATCATCTCAGCCCTAGCAGCAGTCCACCTCCTGTTCCTCCATGAA 120
sbz39al      TTCATCCTTCCATTTATCATCTCAGCCCTAGCAGCAGTCCACCTCCTGTTCCTCCATGAA 120
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bhz23wt      TTCATCCTTCCATTTATCATCTCAGCCCTAGCAGCAGTCCACCTCCTATTCCTCCATGAA 120
bhz28wt      TTCATCCTTCCATTTATCATCTCAGCCCTAGCAGCAGTCCACCTCCTATTCCTCCATGAA 120
bhz22wt      TTCATCCTTCCATTTATCATCTCAGCCCTAGCAGCAGTCCACCTCCTATTCCTCCATGAA 120
bhz20wt      TTCATCCTTCCATTTATCATCTCAGCCCTAGCAGCAGTCCACCTCCTATTCCTCCATGAA 120
bhz63t       TTCATCCTTCCATTTATCATCTCAGCCCTAGCAGCAGTCCACCTCCTATTCCTCCATGAA 120
bhz56t       TTCATCCTTCCATTTATCATCTCAGCCCTAGCAGCAGTCCACCTCCTATTCCTCCATGAA 120
bhz26t       TTCATCCTTCCATTTATCATCTCAGCCCTAGCAGCAGTCCACCTCCTATTCCTCCATGAA 120
bhz30t       TTCATCCTTCCATTTATCATCTCAGCCCTAGCAGCAGTCCACCTCCTATTCCTCCATGAA 120
bhz45t       TTCATCCTTCCATTTATCATCTCAGCCCTAGCAGCAGTCCACCTCCTATTCCTCCATGAA 120
bhz25t       TTCATCCTTCCATTTATCATCTCAGCCCTAGCAGCAGTCCACCTCCTATTCCTCCATGAA 120
dz14sl       TTCATCCTTCCATTTATCATCTCAGCCCTAGCAGCAGTCCACCTCCTATTCCTCCATGAA 120
dz15sl       TTCATCCTTCCATTTATCATCTCAGCCCTAGCAGCAGTCCACCTCCTATTCCTCCATGAA 120
dz16sl       TTCATCCTTCCATTTATCATCTCAGCCCTAGCAGCAGTCCACCTCCTATTCCTCCATGAA 120
gz21cl       TTCATCCTCCCATTTATCATCTCAGCCTTAGCAGCAGTTCACCTCTATTTCTCCATGAA 120
gz22cl       TTCATCCTCCCATTTATCATCTCAGCCTTAGCAGCAGTTCACCTTCTATTTCTCCATGAA 120
chimss       TTTATCTTACCCTTCATTATCACAGCCCTAACAACACTTCATCTCCTATTCTTACACGAA 120
humsk        TTCATCTTGCCCTTCATTATTGCAGCCCTAGCAGCACTCCACCTCCTATTCTTGCACGAA 120
             ** ***  *  **  **  **  **    ****   ** ** * **  ** ** **  *  ** **
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sbz22al      ACAGGATCTAATAACCCCTCAGGAATGGTATCTGACTCAGATAAAATTCCATTCCATCCA 180
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sbz38al      ACAGGATCTAATAACCCCTCAGGAATGGTATCTGACTCAGATAAAATTCCATTCCATCCA 180
sbz39al      ACAGGATCTAATAACCCCTCAGGAATGGTATCTGACTCAGATAAAATTCCATTCCATCCA 180
adil.flesh   ACAGGATCTAACAACCCCTCAGGAATAGTATCCGACTCAGACAAAATTCCATTCCACCCA 180
gz1nl        ACAGGATCTAACAACCCCTCAGGAATAGTATCCGACTCAGACAAAATTCCATTCCACCCA 180
gz2nl        ACAGGATCTAACAACCCCTCAGGAATAGTATCTGACTCAGACAAAATTCCATTCCACCCA 180
gz3nl        ACAGGATCTAACAACCCCTCAGGAATAGTATCTGACTCAGACAAAATTCCATTCCACCCA 180
bhz23wt      ACAGGATCTAACAACCCCTCAGGAATAGTATCTGACTCAGACAAAATCCCGTTCCACCCA 180
bhz28wt      ACAGGATCTAACAACCCCTCAGGAATAGTATCTGACTCAGACAAAATCCCGTTCCACCCA 180
bhz22wt      ACAGGATCTAACAACCCCTCAGGAATAGTATCTGACTCAGACAAAATCCCGTTCCACCCA 180
bhz20wt      ACAGGATCTAACAACCCCTCAGGAATAGTATCTGACTCAGACAAAATCCCGTTCCACCCA 180
bhz63t       ACAGGATCTAACAACCCCTCAGGAATAGTATCTGACTCAGACAAAATCCCGTTCCACCCA 180
bhz56t       ACAGGATCTAACAACCCCTCAGGAATAGTATCTGACTCAGACAAAATCCCGTTCCACCCA 180
bhz26t       ACAGGATCTAACAACCCCTCAGGAATAGTATCTGACTCAGACAAAATCCCGTTCCACCCA 180
bhz30t       ACAGGATCTAACAACCCCTCAGGAATAGTATCTGACTCAGACAAAATCCCGTTCCACCCA 180
bhz45t       ACAGGATCTAACAACCCCTCAGGAATAGTATCTGACTCAGACAAAATCCCGTTCCACCCA 180
bhz25t       ACAGGATCTAACAACCCCTCAGGAATAGTATCTGACTCAGACAAAATCCCGTTCCACCCA 180
dz14sl       ACAGGATCTAACAACCCCTCAGGAATAGTATCTGACTCAGACAAAATCCCGTTCCACCCA 180
dz15sl       ACAGGATCTAACAACCCCTCAGGAATAGTATCTGACTCAGACAAAATCCCGTTCCACCCA 180
dz16sl       ACAGGATCTAACAACCCCTCAGGAATAGTATCTGACTCAGACAAAATCCCGTTCCACCCA 180
gz21cl       ACAGGATCCAATAACCCCTCAGGAATGGTATCTGATTCAGACAAAATCCCGTTCCACCCG 180
gz22cl       ACAGGATCCAATAACCCCTCAGGAATGGTATCCGATTCAGACAAAATCCCGTTCCACCCG 180
chimss       ACAGGATCAAATAACCCCTGGGAATCACCTCCCACTCCGACAAAATTACCTTCCACCCC 180
humsk        ACGGGATCAAACAACCCCCTAGGAATCACCTCCCATTCCGATAAAATCATCTTCCACCCT 180
             ** ***** ** ******    *****   **  * ** ** *****    ***** **

sbz22al      TACTATACAATCAAAGATATCCTAGGCCTTCTAGTACTAATCTTAACACTCATACTACTC 240
sbz38al      TACTATACAATCAAAGATATCCTAGGCCTTCTAGTACTAATCTTAACACTCATACTACTC 240
sbz39al      TACTATACAATCAAAGATATCCTAGGCCTTCTAGTACTAATCTTAACACTCATACTACTC 240
adil.flesh   TACTACACAATCAAAGATATCCTGGGCCTTCTAGTACTAATCCTAGCACTCATACTACTC 240
gz1nl        TACTACACAATCAAAGATATCCTGGGCCTTCTAGTACTAATCCTAGCACTCATACTACTC 240
gz2nl        TACTACACAATCAAAGACATCCTGGGCCTTCTAGTACTAATCTTAGCACTCATACTACTC 240
gz3nl        TACTACACAATCAAAGACATCCTGGGCCTTCTAGTACTAATCTTAGCACTCATACTACTC 240
bhz23wt      TACTACACAATCAAAGACATCCTGGGCCTTCTAGTACTAATCCTAACACTCATACTACTC 240
bhz28wt      TACTACACAATCAAAGACATCCTGGGCCTTCTAGTACTAATCCTAACACTCATACTACTC 240
bhz22wt      TACTACACAATCAAAGACATCCTGGGCCTTCTAGTACTAATCCTAACACTCATACTACTC 240
bhz20wt      TACTACACAATCAAAGACATCCTGGGCCTTCTAGTACTAATCCTAACACTCATACTACTC 240
bhz63t       TACTACACAATCAAAGACATCCTGGGCCTTCTAGTACTAATCCTAACACTCATACTACTC 240
bhz56t       TACTACACAATCAAAGACATCCTGGGCCTTCTAGTACTAATCCTAACACTCATACTACTC 240
bhz26t       TACTACACAATCAAAGACATCCTGGGCCTTCTAGTACTAATCCTAACACTCATACTACTC 240
bhz30t       TACTACACAATCAAAGACATCCTGGGCCTTCTAGTACTAATCCTAACACTCATACTACTC 240
bhz45t       TACTACACAATCAAAGACATCCTGGGCCTTCTAGTACTAATCCTAACACTCATACTACTC 240
bhz25t       TACTACACAATCAAAGACATCCTGGGCCTTCTAGTACTAATCCTAACACTCATACTACTC 240
dz14sl       TACTACACAATCAAAGACATCCTGGGCCTTCTAGTACTAATCCTAACACTCATACTACTC 240
dz15sl       TACTACACAATCAAAGACATCCTGGGCCTTCTAGTACTAATCCTAACACTCATACTACTC 240
dz16sl       TACTACACAATCAAAGACATCCTGGGCCTTCTAGTACTAATCCTAACACTCATACTACTC 240
gz21cl       TACTATACAATCAAAGATATCCTAGGCCTCCTAGTTCTAATTCTAGCGCTCACACTACTT 240
gz22cl       TACTATACAATCAAAGATATCCTAGGCCTCCTAGTTCTAATTCTAGCGCTCACACTACTT 240
chimss       TACTACACAATCAAAGATATCCTTGGCTTATTCCTTTCCTCCTTATCCTAATGACATTA 240
humsk        TACTACACAATCAAAGACGCCCTCGGCTTACTTCTCTTCCTTCTCTCCTTAATGACATTA 240
             ***** ********** *** *** *  *  * *   *  * *      * *     * *

sbz22al      GTCCTATTCTCACCAGACCTATTAGGAGATCCCGACAACTATACCCCCGCCAATCCTCTA 300
sbz38al      GTCCTATTCTCACCAGACCTATTAGGAGATCCCGACAACTATACCCCCGCCAATCCTCTA 300
sbz39al      GTCCTATTCTCACCAGACCTATTAGGAGATCCCGACAACTATACCCCCGCCAATCCTCTA 300
adil.flesh   GTCCTATTCTCACCAGACCTGCTAGGAGACCCCGACAACTACATCCCTGCCAACCCTCTA 300
gz1nl        GTCCTATTCTCACCAGACCTGTTAGGAGACCCCGATAACTACATCCCTGCCAACCCTCTA 300
gz2nl        GTCCTATTCTCACCAGACCTGTTGGGAGACCCCGATAACTACATCCCCGCCAACCCTCTA 300
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gz3nl        GTCCTATTCTCACCAGACCTGTTGGGAGACCCCGATAACTACATCCCCGCCAACCCTCTA 300
bhz23wt      GTCCTATTCTCACCAGACCTATTAGGGGACCCCGATAACTACATCCCCGCCAACCCTCTA 300
bhz28wt      GTCCTATTCTCACCAGACCTATTAGGGGACCCCGATAACTACATCCCCGCCAACCCTCTA 300
bhz22wt      GTCCTATTCTCACCAGACCTATTAGGGGACCCCGATAACTACATCCCCGCCAACCCTCTA 300
bhz20wt      GTCCTATTCTCACCAGACCTATTAGGGGACCCCGATAACTACATCCCCGCCAACCCTCTA 300
bhz63t       GTCCTATTCTCACCAGACCTATTAGGGGACCCCGATAACTACATCCCCGCCAACCCTCTA 300
bhz56t       GTCCTATTCTCACCAGACCTATTAGGGGACCCCGATAACTACATCCCCGCCAACCCTCTA 300
bhz26t       GTCCTATTCTCACCAGACCTATTAGGGGACCCCGATAACTACATCCCCGCCAACCCTCTA 300
bhz30t       GTCCTATTCTCACCAGACCTATTAGGGGACCCCGATAACTACATCCCCGCCAACCCTCTA 300
bhz45t       GTCCTATTCTCACCAGACCTATTAGGGGACCCCGATAACTACATCCCCGCCAACCCTCTA 300
bhz25t       GTCCTATTCTCACCAGACCTATTAGGGGACCCCGATAACTACATCCCCGCCAACCCTCTA 300
dz14sl       GTCCTATTCTCACCAGACCTATTAGGGGACGCCGATAACTACATCCCCGCCAACCCTCTA 300
dz15sl       GTCCTATTCTCACCAGACCTATTAGGGGACGCCGATAACTACATCCCCGCCAACCCTCTA 300
dz16sl       GTCCTATTCTCACCAGACCTATTAGGGGACGCCGATAACTACATCCCCGCCAACCCTCTA 300
gz21cl       GTTCTATTCTCCCCAGACCTACTAGGAGACCCCTGACAATTACACTCCCGCCAACCCTCTA 300
gz22cl       GTTCTATTCTCCCCAGACCTACTAGGAGACCCCTGACAATTACACTCCCGCCAACCCTCTA 300
chimss       ACACTATTCTCACCAGACCTCCTGGGCGATCCAGACAACTATACCCTAGCTAACCCCCTA 300
humsk        ACACTATTCTCACCAGACCTCCTGGGCGACCCAGACAATTATACCCTAGCCAACCCCTTA 300
             ********* ********  *  **  ** *  ** ** ** *   *   ** ** **   **

sbz22al      AGCACCCCTCCCCATATCAAACCTGAAT 328
sbz38al      AGCACCCCTCCCCATATCAAACCTGAAT 328
sbz39al      AGCACCCCTCCCCATATCAAACCTGAAT 328
adil.flesh   AATACCCCTCCCCATATCAAGCCTGAAT 328
gz1nl        AATACCCCTCCCCATATCAAGCCTGAAT 328
gz2nl        AATACCCCTCCCCATATCAAGCCTGAAT 328
gz3nl        AATACCCCTCCCCATATCAAGCCTGAAT 328
bhz23wt      AACACCCCTCCCCATATCAAGCGCGAAT 328
bhz28wt      AACACCCCTCCCCATATCAAGCGCGAAT 328
bhz22wt      AACACCCCTCCCCATATCAAGCGCGAAT 328
bhz20wt      AACACCCCTCCCCATATCAAGCGCGAAT 328
bhz63t       AACACCCCTCCCCATATCAAGCGCGAAT 328
bhz56t       AACACCCCTCCCCATATCAAGCGCGAAT 328
bhz26t       AACACCCCTCCCCATATCAAGCGCGAAT 328
bhz30t       AACACCCCTCCCCATATCAAGCGCGAAT 328
bhz45t       AACACCCCTCCCCATATCAAGCGCGAAT 328
bhz25t       AACACCCCTCCCCATATCAAGCGCGAAT 328
dz14sl       AACACCCCTCCCCATATCAAGCCCGAAT 328
dz15sl       AACACCCCTCCCCATATCAAGCCCGAAT 328
dz16sl       AACACCCCTCCCCATATCAAGCCCGAAT 328
gz21cl       AATACCCCTCCCCATATCAAGCCTGAAT 328
gz22cl       AATACCCCTCCCCATATCAAGCCTGAAT 328
chimss       AACACCCCACCCCACATTAAACCCGAAT 328
humsk        AACACCCCTCCCCACATCAAGCCCGAAT 328
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Table 7 (Tables 7a, 7b, 7c and 7d). The comparison of the molecular signatures of different animal species investigated along with 'adil.flesh', the confiscated skin of unknown animal origin. This table demonstrates the variable positions (i.e. the positions which are not marked with star (*) symbol in Table 6), amongst the 328 bp fragment revealed from the animals listed in Table 5. The dot (.) mark represents the presence of the similar nucleotide as listed in lane 1 i.e. the sequence from "adil.flesh" at that position. It demonstrates that the signatures of each species are unique and specific to its species. The molecular signatures of 'adil.flesh' are comparable (except for position 37 which has a transition from 'T' to 'C') to the molecular signature of 'gz1L' i.e. the known leopard *Panthera pardus* source, indicating the identity of the source of confiscated skin 'adil.flesh' as that of a leopard *Panthera pardus* source. The nucleotide variations (at the positions 153, 198, 223, 264, among the known leopards, (i.e. gz1L, gz2L, and gz3L, respectively)), give an idea about the geographical habitat of each animals. Various studies referring to molecular evolution of different animal species support this hypothesis[75]; however, it could further be confirmed by taking the reference animals from different geographical areas and analyzing by our primers 'mcb 398' and 'mcb869'. If we could generate the database of different haplotypes (i.e. habitat specific molecular signatures) of the animal species, it would also enable our primers to reveal the geographical location of the commitment of wildlife crime.

Table 7a

Position	17	25	29	30	31	33	37	39	48	51	52	57	63	67	69	72	75	78	81	82	87	88	91	94	97	99	102	105	108	111	112
adil.flesh	T	G	A	A	G	T	G	G	C	T	G	C	C	C	T	A	T	C	C	T	T	C	G	G	G	C	C	A	C	C	C
gz1l	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
gz2l	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
gz3l	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
bhz25t	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
bhz26t	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.
bhz30t	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.
bhz45t	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.
bhz25t	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.
bhz20wt	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.
bhz22wt	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.
bhz23wt	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.
dz14sl	.	C	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.
dz15sl	.	C	.	.	.	C	.	.	T	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.
sbz22al	.	.	.	.	.	C	.	.	T	.	C	.	.	.	C	.	.	.	.	.	T	T	.	.	.	.	.	.	.	.	.
sbz38al	.	.	.	.	.	C	.	.	.	.	C	.	.	.	C	.	.	.	.	.	T	T	.	.	.	.	.	.	G	.	.
gz21cl	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	G	.	.
gz22cl	.	.	.	.	.	C	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	T	.	.	.	T	.
chimss	A	.	G	C	C	.	C	.	.	C	A	.	T	T	A	C	C	.	A	C	C	T	A	A	C	T	T	.	.	.	T
hunsk	A	G	T	C	C	.	C	.	.	C	A	.	T	T	G	C	C	T	C	C	C	T	A	A	T	T	T	.	.	.	T

159

Table 7b

Position	114	117	120	123	129	132	139	140	141	147	148	149	150	153	154	156	159	162	168	169	170	171	177	180	186	198	199	200	204	208	210
ad.II.flesh	T	C	G	A	T	C	T	C	A	A	G	T	A	C	G	C	A	C	T	G	C	C	A	C	T	A	T	G	C	T	
gz1l																															
gz2l																															
gz3l	C																														
bhz25t	C	T												T					C			G			C	C					
bhz26t	C	T												T					C			G			C	C					
bhz30t	C	T												T					C			G			C	C					
bhz45t	C	T												T					C			G			C	C					
bhz56t	C	T												T					C			G			C	C					
bhz20wt	C	T												T					C			G			C	C					
bhz22wt	C	T												T					C			G			C	C					
bhz23wt	C	T												T					C			G			C	C					
dz14sl	C	T												T					C			G			C	C					
dz15sl	C	T												T				T	C			G	T	G	C			A	A	C	
sbz22al	C	A		C					G									T					T	G	T			A	A		
sbz38al	C	A		C					G									T					T	G	T			A	A		
gz21cl	C	A		C					G									C						G	C				A		
gz22cl	C	A		C					G							T		C						G	C				A		
chimrss	A	A	G	A			C	T	G	C	A	C	C	C		C	T	C	A	T	C	A	T	C	C	C			A	A	
humsk	G	A	G	A			C	T	T	G	C	A	C	C		C	T	C	A	T	C	A	T	C	C	C	C	G	C	C	T	A

Table 7c

Position	211	213	214	216	217	219	220	222	223	225	226	227	228	229	231	233	234	235	236	238	240	241	242	243	252	261	262	264	267	270	271
adll.flesh	C	A	G	A	C	A	A	C	C	·	·	A	A	C	C	T	A	C	T	C	C	G	T	C	A	T	T	A	A	C	C
gz1l	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
gz2l	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	G	G	·	·	·
gz3l	·	·	·	·	·	·	·	·	T	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	G	·	·
bhz25t	·	·	·	·	·	·	·	·	·	·	A	·	·	·	·	·	·	·	·	·	·	·	·	·	·	A	·	·	G	·	·
bhz26t	·	·	·	·	·	·	·	·	·	·	A	·	·	·	·	·	·	·	·	·	·	·	·	·	·	A	·	·	G	·	·
bhz30t	·	·	·	·	·	·	·	·	·	·	A	·	·	·	·	·	·	·	·	·	·	·	·	·	·	A	·	·	G	·	·
bhz45t	·	·	·	·	·	·	·	·	·	·	A	·	·	·	·	·	·	·	·	·	·	·	·	·	·	A	·	·	G	·	·
bhz56t	·	·	·	·	·	·	·	·	·	·	A	·	·	·	·	·	·	·	·	·	·	·	·	·	·	A	·	·	G	·	·
bhz20wt	·	·	·	·	·	·	·	·	·	·	A	·	·	·	·	·	·	·	·	·	·	·	·	·	·	A	·	·	G	·	·
bhz22wt	·	·	·	·	·	·	·	·	·	·	A	·	·	·	·	·	·	·	·	·	·	·	·	·	·	A	·	·	G	·	·
bhz23wt	·	·	·	·	·	·	·	·	·	·	A	·	·	·	·	·	·	·	·	·	·	·	·	·	·	A	·	·	G	·	·
dz14sl	·	·	·	·	·	·	·	·	·	·	A	·	·	·	·	·	·	·	·	·	·	·	·	·	·	A	·	·	G	·	·
dz15sl	·	·	·	·	·	·	·	·	T	·	A	·	·	·	·	·	·	·	·	·	·	·	·	·	·	A	·	·	G	T	T
shz22al	·	·	·	·	·	·	·	·	T	·	A	·	·	·	·	·	·	·	·	·	·	·	·	·	·	A	·	·	·	·	·
shz38al	·	·	·	·	·	·	·	·	·	·	·	·	G	·	·	C	·	·	·	·	·	·	·	·	·	A	C	C	·	·	·
gz21cl	·	·	·	·	·	·	·	T	T	·	·	·	G	·	·	C	·	·	·	T	T	·	T	C	A	C	C	C	·	·	·
gz22cl	·	·	·	·	T	·	·	T	T	·	·	·	G	·	T	·	·	·	·	·	·	·	·	·	·	A	C	C	·	·	·
chimiss	T	C	C	C	C	A	A	C	T	·	A	·	T	C	·	G	A	C	T	A	A	C	A	C	A	C	C	C	G	C	T
humsk	T	C	C	C	C	T	C	T	C	·	·	·	T	T	·	G	A	C	T	A	A	C	A	C	A	C	C	C	G	C	C

Table 7d

Position	273	276	279	282	284	285	287	288	291	294	297	298	302	303	309	315	318	321	323	324
adil.flesh	C	T	C	C	T	C	C	T	C	C	T	C	A	T	T	T	C	G	C	T
gz1l	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
gz2l	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
gz3l	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.
bhz25t	.	.	.	.	.	.	.	C	C	.	.	.	.	.	.	.	.	.	G	C
bhz28t	.	.	.	.	.	.	.	C	C	.	.	.	.	C	.	.	.	.	G	C
bhz30t	.	.	.	.	.	.	.	C	C	.	.	.	.	C	.	.	.	.	G	C
bhz45t	.	.	.	.	.	.	.	C	C	.	.	.	.	C	.	.	.	.	G	C
bhz56t	.	.	.	.	.	.	.	C	C	.	.	.	.	C	.	.	.	.	G	C
bhz20wt	.	.	.	.	.	.	.	C	C	.	.	.	.	C	.	.	.	.	G	C
bhz22wt	.	.	.	.	.	.	.	C	C	.	.	.	.	C	.	.	.	.	G	C
bhz23wt	.	.	.	.	.	.	.	C	C	.	.	.	.	C	.	.	.	.	G	C
dz14sl	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	C	.
dz16sl	.	.	.	.	.	.	.	C	.	.	.	.	G	C	.	.	.	A	.	.
sbz22al	.	C	.	T	.	C	.	C	.	T	.	.	G	C	.	.	.	A	.	.
sbz38al	.	C	.	C	T	C	.	C	.	.	.	.	.	.	.	.	.	.	.	.
gz21cl	T	C	T	C	.	C	.	C	.	.	.	.	.	.	.	.	.	.	.	.
gz22cl	A	C	C	T	C	.	.	T	A	T	.	T	.	C	A	C	T	A	.	C
chimss	A	C	C	T	T	C	.	T	A	T	C	T	C	A	C	T	A	.	C	C
numsk	A	C	T	A	T	.	.	T	A	.	.	.	C	C	C	C	A	.	.	C

Table 8. Percent similarity matrix calculated by pair-vise comparisons of nucleotide sequences aligned (illustrated in Table 6). The cytochrome b gene sequence of DNA isolated from confiscated material had maximum similarity (99.7% and 98.2%, with the lineages of animals 'gz2L' and 'gz3L', respectively) with the sequences obtained from known normal leopard source, indicating its identity as that of a leopard origin. The similarity matrix has been calculated using the software *PHYLIP (3.5)*.

Table 8. Percent similarity matrix calculated by pair-vise comparisons of cytochrome b gene sequences revealed from 'adil.flesh' and different felids

	bhz20wt	bhz25t	dz14sl	humsk	chimss	bhz22al	gz1L	gz2L	gz3L	gz21cl	adil.flesh
bhz20wt	100	100	99.1	81.7	78.7	93.3	95.1	95.4	95.4	89.6	95.4
bhz25t		100	99.1	81.7	78.7	93.3	95.1	95.4	95.4	89.6	95.4
dz14sl				81.4	78.4	93	94.8	95.1	95.1	89.3	95.1
humsk					86.9	79.6	81.1	80.2	80.2	79	81.4
chimss						78.7	79.6	78.7	78.7	76.8	79.9
bhz22al							92.1	92.4	92.4	89	92.4
gz1L								98.5	98.5	89.3	99.7
gz2L								100	100	88.1	98.2
gz3L									100	88.1	98.2
gz21cl											89.6
adll.flesh											

Table 9. Animals selected for validation of minimum P,S score for efficient amplification of cytochrome b gene of different origin by the primers **'mcb398'** and **'mcb869'**. P,S score of primers **'AFF'** and **'AFR'** for these animals are shown.

Table 9. Animals selected for validation of minimum P'S score for efficient amplification
of DNA templates in PCR

SL.	Name	P, S/AFF	P, S/AFR
1	Indian black buck (Antilope cervicapra)	97, 58	96, 54
2	Sheep (Ovis	87, 53	96, 54
3	Pig (*Sus scrofa*)	87, 52	87, 41
4	Fresh water dolphin (*Platanista gangetica*)	86, 49	82, 47

Table 10. BLAST analysis of primers 'mcb398' in *nr* database of NCBI . It demonstrates that the 3' end of this primer is highly conserved among a vast range of animal species. It also shows the significant homology among the primer and templates (i.e. the cytochrome b gene fragment of different animal species), confirming the universal nature of our primer

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Altschul, Stephen F., Thomas L. Madden, Alejandro A. Sch&auml;ffer,
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  Number of letters in database: 2,863,827,885
  Number of sequences in database:  807,597

Lambda      K        H
    1.37     0.711     1.31

Gapped
Lambda      K        H
    1.37     0.711     1.31


Matrix: blastn matrix:1 -3
Gap Penalties: Existence: 5, Extension: 2
Number of Hits to DB: 39355
Number of Sequences: 807597
Number of extensions: 39355
Number of successful extensions: 15066
Number of sequences better than 10.0: 5706
length of query: 25
length of database: 2,863,827,885
effective HSP length: 17
effective length of query: 8
effective length of database: 2,850,098,736
effective search space: 22800789888
effective search space used: 22800789888
T: 0
A: 30
X1: 6 (11.9 bits)
X2: 15 (29.7 bits)
S1: 12 (24.3 bits)
S2: 16 (32.2 bits)

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Table 11. BLAST analysis of primers 'mcb869' in *nr* database of NCBI. It demonstrates that the 3' end of this primer is highly conserved among a vast range of animal species. It also shows the significant homology among the primer and templates (i.e. the cytochrome b gene fragment of different animal species), confirming the universal nature of our primer.

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<b>BLASTN 2.1.2 [Nov-13-2000]</b>


<b><a href="http://www.ncbi.nlm.nih.gov/htbin-
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Altschul, Stephen F., Thomas L. Madden, Alejandro A. Sch&auml;ffer,
Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997),
"Gapped BLAST and PSI-BLAST: a new generation of protein database search
programs",  Nucleic Acids Res. 25:3389-3402.
<p>
RID: 984593033-24247-14777
<p>
<b>Query=</b>
          (26 letters)


<p>
<b>Database:</b> nt
            807,597 sequences; 2,863,827,885 total letters

<p> <p>If you have any problems or questions with the results of this search
<br>please refer to the <b><a
href=http://www.ncbi.nlm.nih.gov/blast/blast_FAQs.html>BLAST FAQs</a></b><br><p>
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porosus mitochondrial c...   46 3e-04
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cytochrome b (cyt b...    <a href = #1374743> 44</a>  0.001
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spinosissimus mitochondrial...    <a href = #4127373> 44</a>   0.001
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cinereoargenteus cytochrome ...   <a href = #2828646> 44</a>  0.001
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sechurae cytochrome b (c...   <a href = #2828644> 44</a>  0.001
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trochilus mitochondria...   <a href = #9998878> 44</a>  0.001
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complete mitochondrial genome   <a href = #9558343> 44</a>  0.001
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unicolor cytochrome b gene...   44 0.001
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partial mitochondri...    <a href = #3320079> 44</a>   0.001
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sanguinolentus cytochrome ...   <a href = #1256207> 44</a>  0.001
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Gapped
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Matrix: blastn matrix:1 -3
Gap Penalties: Existence: 5, Extension: 2
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Number of sequences better than 10.0: 2441
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length of database: 2,863,827,885
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X2: 15 (29.7 bits)
S1: 12 (24.3 bits)
S2: 16 (32.2 bits)

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Table 12. Other animal belonging to distantly related animal species, investigated to confirm the universal nature of primers **'mcb398'** and **'mcb869'**. Gel photograph showing the PCR amplicons from these animals are shown in Figure 4.

Table 12. The other animals belonging to distantly related species analyzed by our primers to demonstrate its universal nature

SN.	Name of the animal
1.	Indian black buck no.1
2.	Indian black buck no 2
3	sheep
4	pig
5	dog
6	chimpanzee (chimss)
7	human (humsk)
8	Hamster
9	crocodile no1
10	crocodile no2
11	turtle no1
12	turtle no2
13	mouse
14	varanus
15	Naga-naga snake
16	Indian elephant
17	hen
18	dugong
19	lizard
20	weaver bird no1
21	weaver bird no2
22	buffalo no1
23	buffalo no 2